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A novel approach to deliver G6PD diagnosis in remote areas of Bangladesh

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Abstract

Background

WHO recommends testing glucose-6-phopshate dehydrogenase (G6PD) activity before prescribing tafenoquine or primaquine for the treatment of *Plasmodium vivax*. In countries where routine G6PD testing is implemented, testing is decentralized and facilitated through point-of-care diagnosis or patient referral and either approach is challenged by logistics, costs, and poor referral adherence. This pilot study assessed the feasibility and acceptability of a third approach in Bangladesh: centralized G6PD-testing.

Methods

Feasibility was defined as the successful completion of sample-collection, transport, accuracy of laboratory testing, timely return of results, and patient reidentification. Acceptability was defined as the extent of support of participants, health-workers, and policymakers for centralized testing. Blood was collected from febrile patients with suspected malaria and G6PD activity was immediately tested by STANDARD™ G6PD Biosensor (SD BIOSENSOR, South Korea, "Biosensor") as baseline measurement for the feasibility assessment. The remaining blood was shipped to Dhaka for another Biosensor measurement and testing by the reference method spectrophotometry. Biosensor and spectrophotometry results were categorized as deficient, intermediate, or G6PD normal and compared. Quantitative results were complemented by in-depth interviews and focus group discussions with patients, health care providers, and policymakers to explore the acceptability of delaying treatment start for the sake of a G6PD test result.

Results and Discussion

120 febrile patients were enrolled. Biosensor measurements in the field and laboratory did not differ significantly (mean difference: 0.16 U/g Hb, 95% Limit of agreement: − 3.83 to 4.16, p = 0.707), suggesting that transport and delay to testing did not compromise centralized G6PD diagnosis. All participants were reidentified within 48 hours of enrolment. Ten IDIs and two FGDs were conducted, including 12 policymakers, six community health workers and nine patients. All patients interviewed supported G6PD testing, recognizing its role in improving the safety of treatment. Most policymakers and all health workers agreed that primaquine treatment could be delayed by 24–48 hours if centralized testing improved treatment safety and the approach was endorsed by the national malaria elimination program.

Conclusions

This novel approach appeared feasible and acceptable within the study setting. Stakeholders signaled support in centralized G6PD testing, though these findings must be verified in other settings.

Introduction

More than 3 billion people are at risk of *Plasmodium vivax* (*P. vivax*) infection [1]. In contrast to most other human pathogenic malaria parasites, *P. vivax* forms dormant liver stages (hypnozoites) that can relapse weeks to months after a primary infection [2]. The only licensed drugs that kill hypnozoites are primaquine (PQ) and tafenoquine (TQ) [3]. Radical cure of *P. vivax* requires a combination of schizontocidal drugs, such as chloroquine (CQ) or artemisinin combination therapy (ACTs), to kill the symptom causing blood stages (schizonts) and hypnozoitocidal drugs (PQ or TQ) to kill the liver stages that cause relapses. Both PQ and TQ can cause severe hemolysis in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency [4]. G6PD deficiency is among the most common enzymopathies, affecting more than 400 million people worldwide. Vivax malaria treatment is complicated by the fact that the highest prevalences of G6PD deficiency occur in malaria endemic areas[5]. In view of the risk of severe drug induced hemolysis the WHO recommends screening patients with *P. vivax* malaria for G6PD deficiency prior to prescribing either PQ or TQ [6, 7].

The availability of reliable G6PD testing in remote areas, where most vivax malaria patients present, is limited [8]. Consequently, radical cure is frequently under prescribed due to fear of drug induced hemolysis [9]. Decentralized, routine point-of-care G6PD testing is often constrained by supply chain management, limited infrastructure, budgetary constraints, and end user-acceptability [10, 11]. Referring patients for testing to a reference center is an alternative approach, however, findings from Cambodia suggest very poor adherence of patients to referrals of around 50% [8, 12, 13]. Since G6PD activity remains stable for at least seven days if stored at 4°C to 8°C, a further possibility is centralized testing, whereby blood is collected at the point of care and sent for reference testing [14]. This approach has not been assessed to date. It may, however, provide a feasible approach to deliver safe radical cure, especially in remote areas where most

cases occur and, in the future, if incidence of vivax malaria infections decreases and per test costs are likely to rise due to fixed costs for equipment, reagents, and trained workers [15].

Over the last 15 years, the morbidity from malaria in Bangladesh has fallen by 93% and its associated mortality by 94%. In contrast, the proportion of malaria due to *P. vivax* infections has increased from 5% to 20%, highlighting the need to ensure safe and effective radical cure [16]. Identifying patients with malaria in Bangladesh is facilitated by government health workers and staff from non-government organizations (NGOs). Health workers visit presumptive malaria patients at their homes for diagnosis and treatment, or patients attend a local primary health care center where they receive limited diagnosis and symptomatic treatment. Patients diagnosed with *P. vivax* malaria are treated with three days CQ (25mg base / kg) plus 14 days of PQ (3.5mg / kg) [17]. Although the prevalence of G6PD deficiency in Bangladesh's malaria hotspots is 9.0%, routine G6PD testing is not part of the national malaria treatment guidelines [4, 17, 18]. The objective of this pilot study was to assess the feasibility and acceptability of centralized G6PD testing in Bangladesh among patients, healthcare providers, and policymakers.

Methods

Definition of Feasibility and Acceptability

Feasibility was defined as the extent to which centralized G6PD testing could be implemented under field conditions, including successful sample collection, transport, laboratory testing, timely return of results, patient reidentification, and the practical capacity of health workers and the health system to support these steps. Acceptability was defined as the extent to which patients, health care providers, and policymakers considered centralized G6PD testing, including venous blood collection, delayed primaquine initiation, communication of results, and follow-up for treatment delivery, to be appropriate and acceptable within the local care context.

Study Setting and Procedures

The study was conducted in the Chittagong Hill Tracts (CHT) in the southeast of Bangladesh (Fig. 1), in one Upazila Health Complex and seven health centers (six government clinics and one NGO)[19]. Staff at each clinic include a community health care provider (CHCP), a health assistant and a family welfare assistant collectively defined as Community Health Worker (CHW). These clinics are located more than 100 kilometers from the nearest tertiary hospital. Residents of the CHT attend government and non-government health care centers for diagnosis and treatment of malaria at no cost. NGO staff also visit residents on a regular basis and offer malaria diagnosis and treatment at home, free of charge [20, 21].

This observational pilot study used a mixed-methods approach (QUAN + QUAL; Fig. 2) [22]. The qualitative component of the study followed the standard consolidated criteria for reporting qualitative research (COREQ) guidelines [23]. Prior to patient enrolment, procedures for identification of participants, sample collection, shipment of samples to a reference center, and returning test results from the reference center were established in collaboration with local health care providers in pre-enrollment workshops. During these workshops, all government and NGO health workers also received comprehensive training on measuring G6PD activity at the point of care.

QUAN: quantitative; QUAL: qualitative; RDT: rapid diagnostic test; G6PD: glucose-6-phopshate dehydrogenase; CHCPs: community health care providers; FGD: focus group discussions; IDIs: in-depth interviews

Patient enrolment, blood collection, G6PD measurement and malaria diagnosis and treatment

All residents of the study area, at least 12 years of age, with axillary temperature ≥ 37.5°C, identified by a local CHW during home visit or when presenting to one of the health care centers, were asked to participate. Following written informed consent, 3 mL of venous blood were collected at a local clinic, and an aliquot was immediately used for a malaria rapid diagnostic test (FalciVax™, Zephyr Biomedicals, India; "mRDT"), G6PD measurement using a Biosensor (STANDARD™ G6PD test, SD Biosensor, Republic of Korea; "Biosensor"), and preparation of two malaria microscopy slides. The remaining sample was shipped to a reference laboratory in Dhaka (around 350 km, approximately 9 hours by public bus) in a foam box at a controlled temperature of 4–8°C. At the reference center in Dhaka, all samples were tested again by Biosensor and the reference method spectrophotometry. Results of both tests were reported back to the CHW by phone.

Testing with the Biosensor was performed according to manufacturer recommendation and results were recorded in U/gHb [24]. All results were then categorized according to manufacturer recommendation: females were classified as deficient (≤ 4 U/gHb), intermediate (> 4 U/gHb to ≤ 6 U/gHb), or normal (> 6 U/gHb), while males were classified as deficient (≤ 4 U/gHb) or normal (> 4 U/gHb) [25].

The reference method spectrophotometry was performed on a UV-Vis Spectrophotometer UV-1900i (Shimadzu Corporation, Japan) using kits from Pointe Scientific (USA; Cat. No.: G7583). Enzyme activity was determined from the change in absorbance at 340 nm at 37°C and normalized by hemoglobin level measured on a Sysmex XP-300 analyzer (Sysmex, Japan). The adjusted male median (AMM) was adapted from a previous survey using the same spectrophotometry procedures on samples from the same population and was defined as 100% activity [18]. Females were classified as deficient (≤ 30% activity of the AMM), intermediate (> 30% to ≤ 70% activity of the AMM), or normal (> 70% activity of the AMM), while males were classified as deficient (≤ 30% activity of the AMM) or normal (> 30% activity of the AMM).

Malaria blood films were assessed in all participants. Thick and thin films were stained with Giemsa and read according to standard procedures by a trained microscopist [26].

All patients were treated according to national guidelines based on the diagnosis provided by the CHW [17]. Patients diagnosed with *P. vivax* malaria by mRDT were treated with CQ for three days (10 mg/kg/day for the first two days and 5 mg/kg/day on the third day) to clear the asexual parasitaemia. PQ was withheld until the results of the G6PD testing were available from the reference center. All G6PD normal patients were treated with 0.25 mg/kg/day PQ for 14 days, G6PD intermediate and deficient patients received weekly doses of 1.0 mg/kg/week for eight weeks[17].

Focus group discussions and in-depth interviews

Two junior qualitative researchers (HF and AZ) conducted the qualitative exploration under the guidance of two senior social scientists with extensive experience in qualitative methodology (NA and BA). The research team members drafted the interview and discussion guides based on the study objectives described in the study protocol. The guidelines were shared with the multidisciplinary group of experts, including clinicians, epidemiologists, public health experts, and social scientists, for their review. Subsequently, the interview guide was piloted with a social scientist (BA) and was revised based on the received feedback. Among the study population, a subset of patients and healthcare providers, including CHWs and laboratory technicians were invited to participate in focus group discussions (FGDs) around the feasibility and acceptability of centralized G6PD testing.

Patients were selected based on the maximal variation sampling to accommodate the maximum diversity of ethnic groups from the first 90 enrolled febrile participants. CHWs were selected purposively, based on willingness to participate and the recommendation of the local Upazila Health and Family Planning officer. Policy decision makers were selected based on malaria-related expertise and experience, current professional engagements under the national malaria elimination program, and job locations i.e. working in urban, rural, and remote areas. Thereby, identified policy decision makers were invited to IDIs and group discussions to assess their willingness to adopt centralized testing and to identify key barriers to rollout. In some cases, follow-up interviews with policy makers and healthcare providers were conducted via mobile phone to clarify identified uncertainties. All respondents were approached for interviews and discussions at their convenient time and location by a research team member. Before conducting the interviews or discussions, the interviewers and moderators briefly introduced themselves and provided a brief overview of the study objectives. The interviews were conducted in Bengali (the local language) and lasted 40 to 90 minutes.

The interviews and discussions were complemented by informal observations and conversations at a community clinic located in Rowangchhari upazila to examine how the healthcare provider approached community members for G6PD testing, responses of community members to G6PD testing, and challenges encountered during the G6PD testing procedure by the community healthcare providers. The qualitative team conducted an informal observation at a community clinic in Rowangchhari upazila to examine how the healthcare provider approached community members for G6PD testing (five individual tests), the reactions of community members, and the challenges encountered by the provider during the testing procedure. The observer took notes on major issues and important observations, which supplemented the data analysis.

Data Analysis

Summary statistics and the proportion of participants who could be correctly re-identified were presented descriptively. The Shapiro-Wilk test was used to assess the normality of the data. Agreement between Biosensor field and laboratory measurements were assessed using the Wilcoxon signed-rank test for paired medians and McNemar's test for paired categorical results. The correlation between Biosensor and spectrophotometry readings was assessed by calculating the Spearman's rank correlation coefficients (r_s) and the same method was also used to assess the correlation between time to testing and measured G6PD activity as a proxy for the impact of transport on enzyme activity. Bland-Altman plots were constructed to assess mean differences and 95% limits of agreement (LoA) between paired Biosensor measurements. To calculate the performance of the Biosensor against the reference method spectrophotometry, a true positive result was defined as a deficient result by Biosensor and spectrophotometry. Performance was calculated stratified by sex considering the

spectrophotometry measurement as reference. The analysis was done twice in females, first considering intermediate results as deficient and then again as G6PD normal. Sensitivity, specificity, and 95% confidence intervals (CI) were calculated using standard formulae and the calculated performance of the Biosensor in the field and laboratory was compared with the DeLong's test [27, 28]. Areas under the curve for Biosensor performance when executed in the field and laboratory were calculated and compared to assess differences in performance as a proxy for end-user competence. All statistical tests were two-sided, and a p-value of < 0.05 was considered statistically significant. All analyses were done using Stata software, version 15.0 (Stata Corporation, College Station, TX, USA).

IDIs and FGDs were transcribed verbatim in Bengali and later translated to English. The team expanded all field notes from informal observations and conversations. The researchers then read the transcripts line by line in an iterative manner. All data were coded manually. Two qualitative research team members (HF and AZ) reviewed transcripts and notes carefully to synthesize codes following a hybrid approach that included inductive and deductive processes. Finally, coding landscapes were discussed among coders and interviewers, reviewed by the third member (NA). The codes were discussed to merge and categorize them into themes and sub-themes. The final themes and sub-themes were constructed based on their relevance to the study objective and the analysis of these themes was conducted using the six-phase approach developed by Braun and Clarke [29]. Four major themes were identified from the coded data: i) Perceptions of patients towards G6PD testing and delayed treatment; ii) Perceptions and experiences of healthcare providers using the Biosensor and delayed treatment; iii) Operational feasibility of introducing point-of-care G6PD testing; and iv) Policy and systems readiness towards integrating G6PD testing

Sample Size

The sample size was calculated to assess differences in measured G6PD categories (deficient, intermediate, normal) between Biosensor readings at the field site compared to Biosensor readings conducted at the reference center. Assuming population proportions of 9.0% deficient, 13.3% intermediate, and 77.3% normal under Hardy–Weinberg equilibrium, and an expected discordance rate of 10%, 108 participants were required using the McNemar–Bowker test for correlated proportions ($\alpha = 0.05$, power = 80%, effect size $w = 0.3$) [18]. Allowing for approximately 10% data loss, the final target sample size was increased to 120 participants. For the qualitative component, enrolment of potential participants continued until no novel findings were found from further interviews aligning with the tenet of data saturation in qualitative methods [30].

Ethical approval

This study was approved by the Ethical Review Committee of icddr,b, Mohakhali, Dhaka (PR- 25017) and the Northern Territory Health and Menzies School of Health Research's Human Research Ethics (NT HREC Reference Number: 2024-5016). All participants or their legal guardians provided written informed consent prior to enrollment, with written assent collected from minors aged 12 years and above.

Results

Pre-enrolment workshops: detailed study procedures

Prior to enrolment, two workshops were held on 29 June 2025 and 10 July 2025, with a total of 18 participants, including national policymakers (n=4), local policy implementers (n=8), and CHWs (n=6). At the end of the second day, it was agreed that all samples would be labeled by a unique numerical identifier and date of blood collection; all samples would be stored in commercially available Styrofoam boxes that would be sealed with tape. Sample shipment to the reference center would be facilitated using the public bus system, feedback would be provided by mobile phone for non-malaria patients and in person for all participants with malaria diagnosis. Irrespective of malaria diagnosis a hard copy of the G6PD result would be provided to each participant for future reference which participants contacted by phone could collect from the health care center. A list with identifiers and names would be kept under lock and key by the local CHW. It was also agreed that every CHW would undergo at least two training sessions before starting to use the Biosensor and results of the field measurement would not be communicated to patients (unless requested), considering the experimental nature of the approach. Results from the reference center would be communicated to each patient, deficient and intermediate patients would be offered counselling by the local medical staff.

Participant enrolment

Between 12 July and 24 September 2025, a total of 120 febrile patients were enrolled into the study. 50.8% (n=61) were male, and the median age was 34.5 years (interquartile range [IQR]: 20–50 years; range: 12–81 years). Between 29 July and 22 September 2025, a total of 27 participants took part in ten IDIs (eight individual IDIs and two IDIs with two participants each) and two FGDs (six and nine participants, respectively), including 12 policymakers, six CHWs (five CHCPs and one lab technician) and nine patients. One field observation (covering five Biosensor measurements) was conducted to document biosensor-based G6PD testing in the field. (Fig 2, Table 1).

Table 1 Demographic characteristics of the qualitative study participants in Dhaka and Bandarban (n=27).

Characteristics	Policymakers (n=12)	CHWs (n=6)	Patients (n=9)
	n (%)	n (%)	n (%)
Male	10 (83.3)	2 (33.3)	5 (55.6)
Female	2 (16.7)	4 (66.6)	4 (44.4)
Median age (years) [IQR, range]	50.0 (47.0 – 57.5)	39.5 (38.0 – 42.0)	30.0 (21.0 – 35.0)
Median working experience (years) [IQR, range]	15.5 (9.5 – 24.0)	14.0 (14.0 – 14.0)	---
Ethnicity			
Bengali	9 (75.0)	0 (0.0)	0 (0.0)
Tangchangya	0 (0.0)	4 (66.7)	4 (44.4)
Marma	2 (16.7)	1 (16.7)	2 (22.2)
Bawm	0 (0.0)	0 (0.0)	3 (33.3)
Chakma	1 (8.3)	0 (0.0)	0 (0.0)
Khiyang	0 (0.0)	1 (16.7)	0 (0.0)

Feasibility

Feasibility of sample collection and transport

Most respondents considered community clinics to be an effective point of contact for facilitating community-based G6PD testing. Community clinic healthcare providers possessed detailed social knowledge and were familiar with households in their catchment areas, which could facilitate patient identification, follow-up, and treatment. All patients reported that accessing care through community clinics was convenient, as these facilities were located nearby and clinic staff were trusted members of the community. One patient noted that if an unfamiliar healthcare worker requested venous blood samples, it could lead to misunderstandings or resistance, whereas the same request made by a known staff member would likely be accepted without objection.

All blood samples collected at the health center were shipped successfully. Paired Biosensor readings were available for 119 patients (99.2%), and all collected samples underwent G6PD testing by spectrophotometry. The median time to testing at the reference laboratory was 1 day (range: 1–2 days) for both spectrophotometry and Biosensor assays.

There was no significant difference (p=0.707) in the median G6PD activities measured in the field (8.7 U/gHb; IQR: 6.8–10.4) and the laboratory (8.6 U/gHb; IQR: 7.3–10.0) and activities measured by Biosensor and spectrophotometry in the laboratory did not correlate with duration of transport (r_s = 0.08, p = 0.409, and r_s = 0.06, p = 0.518, respectively) (Fig 3).

Feasibility of Biosensor testing

While government and NGO officials were confident that CHWs could manage tasks such as malaria rapid diagnostic tests (mRDTs), collecting venous blood could be challenging and should be facilitated by better qualified staff such as laboratory technicians. A technician at the sub-district healthcare center confirmed that collecting venous blood and conducting the Biosensor was not a difficult task for them, as they had received professional training in blood collection. In contrast all five CHCPs stated that they were not trained to collect venous blood and felt this would remain a challenge even with additional training. At a community clinic, a CHCP was observed to perform the Biosensor according to standard procedures but did not check expiry dates. During one attempt, they struggled to set the machine up correctly and execute test procedures according to the manual. They highlighted feeling nervous due to the novelty of the test. One CHW reported:

"I feel nervous, since this is my first time conducting the G6PD test. I believe it will be fine after getting some experience." ***– 38-year, female, CHW***

These observations and the CHW sentiment were partially confirmed by the quantitative findings. There was a significant and positive correlation between Biosensor measurements in the field and laboratory (r_s = 0.73, p < 0.001), between Biosensor measurements in the field and spectrophotometry (r_s = 0.72, p < 0.001), as well as Biosensor measurements in the laboratory and spectrophotometry (r_s = 0.80, p < 0.001) (Fig. 4, S1A, and S2A). However, in males, the sensitivity of the Biosensor in the field was 80.0% (95% CI: 28.4–99.5) compared to 100.0% (95% CI: 47.8–100.0) in the laboratory, while specificity did not change and was 100.0% (95% CI: 93.6–100.0) in the field and 100.0% (95% CI: 93.5–100.0) in the laboratory. In females, sensitivity and specificity at the 4U/gHb threshold were 100.0% (95% CI: 2.5–100.0 and 93.8–100.0, respectively) in both field and laboratory measurements. When the higher threshold of 6U/gHb was applied in samples from females, the sensitivity was100.0% (95% CI: 2.5–100.0) in both settings, while specificity decreased to 87.9% (95% CI: 76.7–95.0) in the field and 93.1% (95% CI: 83.3–98.1) in the laboratory (Table 2).

Table 2 Performance of the Biosensor in field and laboratory for detecting G6PD deficiency and/or intermediate compared with the reference spectrophotometry

Biosensor Threshold	**Sensitivity (95% CI)**	**Specificity (95% CI)**	**p-value**
≤4 U/gHb	**Male**		
Field	80.0 (28.4 – 99.5)	100.0 (93.6 – 100.0)	0.317
Laboratory	100.0 (47.8 – 100.0)	100.0 (93.5 – 100.0)	
≤4 U/gHb	**Female**		
Field	100.0 (2.5 – 100.0)	100.0 (93.8 – 100.0)	---
Laboratory	100.0 (2.5 – 100.0)	100.0 (93.8 – 100.0)	
≤6 U/gHb			
Field	100.0 (2.5 – 100.0)	87.9 (76.7 – 95.0)	0.177
Laboratory	100.0 (2.5 – 100.0)	93.1 (83.3 – 98.1)	

Categorical results of the Biosensor did not differ between field and laboratory for males (p=0.317) and females (p=0.180). A total of 5 (4.2%) patients were diagnosed as G6PD deficient by Biosensor in the field, and a sixth G6PD deficient individual was identified by the Biosensor in the laboratory measurement and confirmed by spectrophotometry (6/119; 5.0%). Four out of seven females identified by the Biosensor field measurement as intermediate were categorized as normal by the Biosensor laboratory measurement; when considering spectrophotometry as reference method, only two out of the seven intermediate females were confirmed. One female identified as G6PD normal by the field Biosensor was categorized as intermediate by both the laboratory Biosensor measurement in the laboratory and spectrophotometry (Table 3 and Table S1).

Table 3 Comparison of G6PD categories by Biosensor (field and laboratory) and spectrophotometry stratified by biological sex. Numbers in brackets are proportions in %.

	Male					Female					
G6PD measurement by	**G6PD activity**	**≤4U/gHb**	**> 4U/gHb**	**Total**	**p-value**	**G6PD activity**	**≤4U/gHb**	**>4 to ≤6 U/gHb**	**> 6U/gHb**	**Total**	**p-value**
	Biosensor in the field										
Biosensor at laboratory	**≤4U/gHb**	4 (6.7)	1 (1.7)	**5**	0.317	**≤4U/gHb**	1 (1.7)	0 (0.0)	0 (0.0)	**1**	0.180
						>4 to ≤6 U/gHb	0 (0.0)	3 (5.1)	1 (1.7)	**4**	
	> 6U/gHb	0 (0.0)	55 (91.7)	**55**		**> 6U/gHb**	0 (0.0)	4 (6.8)	50 (84.7)	**54**	
	Total	**4**	**56**	**60**		**Total**	**1**	**7**	**51**	**59**	
	Biosensor in the field										
Spectrophotometry at laboratory	**≤30% of AMM**	4 (6.6)	1 (1.6)	**5**	0.368	**<30% of AMM**	1 (1.7)	0 (0.0)	0 (0.0)	**1**	0.102
						≥30% to ≤70% of AMM	0 (0.0)	2 (3.4)	1 (1.7)	**3**	
	>30% of AMM	0 (0.0)	56 (91.8)	**56**		**>70% of AMM**	0 (0.0)	5 (8.5)	50 (84.7)	**55**	
	Total	**4**	**57**	**61**		**Total**	**1**	**7**	**51**	**59**	
	Biosensor in the laboratory										
Spectrophotometry at laboratory	**≤30% of AMM**	5 (8.3)	0 (0.0)	**5**	0.317	**<30% of AMM**	1 (1.7)	0 (0.0)	0 (0.0)	**1**	0.564
						≥30% to ≤70% of AMM	0 (0.0)	2 (3.4)	1 (1.7)	**3**	
	>30% of AMM	0 (0.0)	55 (91.7)	**55**		**>70% of AMM**	0 (0.0)	2 (3.4)	53 (89.8)	**55**	
	Total	**5**	**55**	**60**		**Total**	**1**	**4**	**54**	**59**	

AMM = 7.03 U/gHb (100% activity)[18]

The observed lower performance in the field was underlined by government and NGO officials, who agreed that the educational background and skills of CHWs were insufficient to handle complex technical procedures, such as performing the Biosensor. A district-level government official said,

"The complicated thing is that the educational background of our field health workers is not sufficient. They are mostly living in different paras [local communities]. I mean, they are from the local communities, and not adequately trained to perform the G6PD test." **–37-year, male, policymaker**

Several government and NGO officials also expressed concerns regarding workload of local staff. Routine tasks of laboratory technicians include microscopic tests and other pathological tests, and CHWs are responsible for implementing various programs related to malaria, tuberculosis, immunization, nutrition, and social welfare in the community. Adding the Biosensor to their duties, along with the need for repeated patient visits, would further heighten their workload, which might not be feasible given their current financial compensation. Some policy decision makers and healthcare providers also mentioned that it would be challenging for CHCPs to send samples to a reference laboratory due to the current high workload. A subdistrict-level government official said that,

"Shipping samples from the community clinic to Bandarban and from Bandarban to Dhaka is possible; however, this process can be costly and time-consuming. There is also a challenge regarding who will be responsible for shipping the blood samples to the sub-district hospital [from the community clinic], as the community clinics lack the necessary manpower. Additionally, if blood samples need to be sent from the sub-district hospital to Bandarban on a daily basis, it will require a dedicated person to manage this task regularly. We also need to cover the

*transportation costs for this person, which is another major concern. Furthermore, the quality of the samples may be affected by this transportation process."****–38-year, female, policymaker***

Feasibility of reporting results back to the field and re-identifying patients

All CHWs reported receiving G6PD test results from the reference center within 24–48 hours, and all patients were successfully reidentified. In the qualitative assessment CHWs provided details on the patients they cared for during the study period. They explained that after obtaining the results, they contacted all enrolled participants through phone calls. The CHW visited participants' homes if patients had not received the call or could not be reached over phone. CHWs considered this process manageable within the study setting. When asked for potential risks to reliable reidentification, CHWs mentioned possible challenges in conducting follow-up visits in border areas, conflict zones, and remote locations, where patient mobility and limited mobile network coverage often hinder communication. CHWs also highlighted the possibility that patients might migrate to different areas after receiving treatment, complicating a reidentification. Most healthcare workers also felt that patients diagnosed with *P. vivax*, would actively contact them again for PQ, if hypnozoitocidal treatment was delayed, being aware of the need for treatment.

Two patients were *P. vivax* positive by mRDT and microscopy and one patient with *P. falciparum* was only positive by microscopy. All malaria-positive patients were classified as G6PD normal by both Biosensor measurements (in the field and the reference laboratory) and spectrophotometry. Participants with *P. vivax* infection were treated with CQ immediately and PQ was administered 24 hours later after G6PD status was confirmed by spectrophotometry.

Acceptability

Patient views

All patients interviewed were supportive of G6PD testing if it would improve safety of radical cure. Patients were aware of the risks and benefits of PQ and that G6PD testing could mitigate these risks. A patient said,

"While sharing my [G6PD] result, they said that I had an anemic issue [G6PD deficiency]. I should take primaquine as advised by the doctors of Sadar [government] hospital. They would determine the dosage of primaquine." ***– 20-year, male, student***

Patients reported that it would not be a major issue if their healthcare provider explained the reasons for delays in PQ treatment during the sample collection, as was done in this pilot study. A patient said,

*"I think the way they [provider] sensitized a patient was very effective. At the beginning, the provider tried to explain why I should do the test. He explained that this test result would help to guide them [doctor/provider] on how and when I should take the malaria medicine. I thought this approach would be very good if we started the treatment more accurately. Then, I showed my interest and agreed to conduct the test."****– 30-year, male, service***

Health Care Provider and Policymaker views

Most policymakers and all CHWs expressed that G6PD diagnosis and PQ administration for *P. vivax* malaria could be delayed for 24 to 48 hours. If the national program were to administer PQ only after determining G6PD status, the CHWs could adhere to this protocol. However, some policymakers mentioned that delayed PQ treatment might cause confusion among patients, as they were typically treated with CQ and PQ immediately after malaria diagnosis, and a multi-step approach might result in patient attrition. The same policy makers also raised concerns that revisiting the healthcare center to collect G6PD reports and PQ dosages could create additional hassle for patients, which might have a negative impact on patient compliance and PQ treatment adherence. A senior program manager of an NGO said:

*"The patient may feel insecure about their treatment. We typically provide them with a card that specifies how many days they will receive chloroquine and primaquine. So, the patient might ask questions such as, "Why aren't you giving me that medicine [primaquine] now?" or "Why should I take that medicine later?" It is important to clearly explain the matter [reasons for delaying primaquine] to the patient."****– 48-year, male, policymaker.***

Policymakers shared that incorporating the G6PD test into the national malaria treatment guidelines could be linked to the arrangement of essential logistics, such as biosensor machines and kits, other necessary supplies, the hiring of new staff, and compensation for field personnel. However, budget constraints within the existing malaria elimination program rendered the introduction of new components challenging. A district-level government official said:

"Here, funding is a big issue. If we want to provide the device [Biosensor] for G6PD testing to all the healthcare providers, it will require huge funding. That is why it has not yet been considered [by policymakers]." ***– 37-year, male, policymaker***

All policymakers felt that crucial information was missing to make an informed decision on the introduction of routine G6PD testing. Key areas included the benefits of routine G6PD testing, the local prevalence of G6PD deficiency within malaria endemic areas of Bangladesh, and the cost-effectiveness of routine testing compared to the current practice of treatment (without G6PD test). Some suggested that since G6PD levels did not change throughout a person's lifetime, a Management Information System (MIS) could be developed to maintain patient records. An official from the NMEP of Bangladesh said:

"Advocate with evidence. Creating evidence is now a priority. If you generate evidence, we can advocate with the policymakers and the technical experts." ***– 53-year, male, policymaker***

Discussion

In this pilot study, centralized G6PD testing appeared feasible and acceptable with two reservations: (1) that procedures and their rationale are clearly explained to patients and community health workers, and (2) that additional evidence is generated to support broader implementation.

Figure 5 summarizes the key aspects that were found to impact on the feasibility and acceptability of centralized testing.

Patient and Health Worker Perspectives

All patients interviewed were supportive of the inclusion of G6PD testing into routine care to improve treatment safety, although only two patients with *P. vivax* were enrolled. Both patients received CQ immediately after malaria diagnosis, while standard low dose PQ treatment for 14 days was provided within 48 hours. Since symptoms typically resolve soon after blood stage CQ treatment has commenced, the delay in PQ treatment may have reduced patients' willingness to complete the subsequent 14-day PQ regimen. A recent study from India reported completion rates of less than 60% for the 14-day PQ regimen, with 62% of patients who did not complete treatment citing symptom resolution as the primary reason for non-adherence [31]. However, participants in this study emphasized that delayed treatment was acceptable if the rationale was clearly explained, suggesting that communication may mitigate potential negative effects of delayed treatment start on adherence. Indeed, adherence to the 14-day PQ regimen is insufficient in approximately 40% of patients (when provided at diagnosis) and poor adherence is associated with a 2.3-fold increase in relapse risk within 90 days [32], highlighting a potential trade-off between timely treatment initiation and improved diagnostic assurance when treatment is guided by confirmed G6PD results.

At the point of first contact, patients appeared receptive to G6PD testing when the purpose and implications of the procedure were clearly explained. Observations and interviews indicated that community health care providers were able to communicate the rationale for testing and the need to delay primaquine treatment in a way that was understood and accepted by patients, while patients emphasized that understanding the link between testing and treatment safety influenced their decision to participate. Clear and comprehensive communication with patients is not only relevant for the acceptability of G6PD testing and delayed PQ treatment but is a known predictor of PQ treatment adherence, underlining the importance of clear and informed communication at the point of first contact [31]. Despite their important role in patient identification, communication and follow up, CHWs expressed hesitation about performing Biosensor testing themselves and preferred a laboratory-based implementation. This is consistent with previous findings describing concerns about procedural complexity, test performance, and the need for training and supervision among community-based health workers [11].

Operational Feasibility

Sample transport and delay to testing did not alter G6PD activity substantially in this setting. At the same time, most respondents considered community clinics to be an effective point of contact for patient identification, treatment provision, and follow up. These findings support a model where community clinics serve as the entry point for enrolment and blood collection, while G6PD testing is performed at higher-level laboratories. Such an approach could preserve the accessibility and trust of community-based care while reducing the risk of misclassification associated with field-based testing.

The Biosensor employed has previously demonstrated very good performance and repeatability and the manufacturer suggested universal cut-offs appear to define G6PD deficiency well, supporting its role for wider roll-out [24, 25, 33]. In contrast to the gold standard spectrophotometry, the device is easier to use, the turnaround is faster and the interpretation is easier [15]. However, reliable use still depends on procedural competence and test procedures require good pipetting skills, found to be challenging for non-laboratory staff in Cambodia [31]. Policy makers and CHWs echoed concerns that field staff might not be suitably qualified for G6PD testing, and testing would be better placed in the hands of well-trained laboratory technicians. Indeed, correct identification of individuals with G6PD deficiency

is essential to prevent drug-induced hemolysis associated with 8-aminoquinoline antimalarials such as PQ and TQ [7]. In this study, clinically relevant discordances were observed between bedside Biosensor results and laboratory-based measurements, including a deficient individual classified as G6PD normal at the bedside and several females classified as intermediate in the field who were subsequently categorized as normal on repeat laboratory testing (Table 2). Given that spectrophotometry is the WHO-recommended reference method performed under controlled conditions by trained personnel, these findings raise concern about misclassification under field conditions, including false normal results in G6PD-deficient individuals.

Policy and System Readiness

Policymakers were open to considering centralized G6PD testing if further operational and economic data became available. Policymakers from a number of settings have underlined the necessity of credible research on diagnostic performance, user adherence, implementation issues, and total economic impact to assist informed policy decisions and promote effective integration into health systems [35-37]. Concerns remained regarding the potential costs of devices (US$ 171–832), test strips (US$ 5–10), and associated consumables[34], as well as the increased workload of staff involved that could not be handled by CHCP. Comprehensive cost-effectiveness evaluations are therefore needed before large-scale implementation, including considerations for quality assurance systems and the ongoing training of laboratory technicians as part of G6PD screening programs[34].

Limitations

Several limitations must be acknowledged. The sample size was small and only two patients with *P. vivax* malaria were included. The study was conducted in a single location, so generalizability may be limited particularly with regards to other endemic settings with different infrastructure and population structure. Cost-effectiveness was not evaluated, resulting in a significant evidence gap for policy adoption. The study demonstrates proof of principle, but its applicability to standard medical practice and different settings is yet to be determined. Finally, delayed PQ treatment may have a negative impact on adherence, this phenomenon was not assessed in this study and requires further investigation in bigger implementation trials.

Conclusion

In remote settings centralized G6PD testing using sample shipment has the potential to be a pragmatic alternative to decentralized testing, maintaining diagnostic integrity whilst reducing reliance on complex point-of-care procedures. Similar sample referral approaches are already being used in other resource-limited settings to link peripheral facilities with higher-level laboratories and expand access to diagnostic testing. However, their success depends on broader health system factors, including laboratory capacity, workforce availability, supply chain reliability, community engagement, and long-term sustainability [38]. As shorter-course, higher-dose PQ regimens and single dose TQ treatment are increasingly being considered, the clinical importance of accurate and robust G6PD diagnosis becomes essential. In this context, approaches that remain operationally and economically viable even when malaria incidence declines are particularly valuable. Further work is warranted to investigate the cost-effectiveness and treatment adherence in a range of settings and explore the role of centralized testing within national malaria elimination strategies in different endemic settings.

Abbreviations

ACTs
artemisinin combination therapy
AMM
adjusted male median
CC
Community Clinic
CHCP
community health care provider
CHT
Chittagong Hill Tracts
CHW
Community health worker
CI
Confidence Interval
COREQ

COnsolidated criteria for REporting Qualitative research
CQ
Chloroquine
FGD
Focus Group Discussions
G6PD
glucose-6-phosphate dehydrogenase
IDI
In-Depth Interviews
IQR
Interquartile Range
LoA
Limits of Agreement
MIS
Management Information Systems
mRDT
Malaria Rapid Diagnostic Test
NGO
Non-Government Organization
NMEP
National Malaria Elimination Program
PQ
Primaquine
QUAL
Qualitative
QUAN
Quantitative
TQ
Tafenoquine
WHO
World Health Organization

Declarations

Ethical approval

This study was approved by the Ethical Review Committee of icddr,b, Mohakhali, Dhaka (PR- 25017) and the Northern Territory Health and Menzies School of Health Research's Human Research Ethics (NT HREC Reference Number: 2024–5016). All participants or their legal guardians provided written informed consent prior to enrollment, with written assent collected from minors aged 12 years and above.

Participant enrolment

Between 12 July and 24 September 2025, a total of 120 febrile patients were enrolled into the study. 50.8% (n = 61) were male, and the median age was 34.5 years (interquartile range [IQR]: 20–50 years; range: 12–81 years). Between 29 July and 22 September 2025, a total of 27 participants took part in ten IDIs (eight individual IDIs and two IDIs with two participants each) and two FGDs (six and nine participants, respectively), including 12 policymakers, six CHWs (five CHCPs and one lab technician) and nine patients. One field observation (covering five Biosensor measurements) was conducted to document biosensor-based G6PD testing in the field. (Fig. 2, Table 1).

Competing interests

The authors declare that they have no competing interests

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Author Contribution

MSH, BL, NAR and MSA conceived the study. MSH, BL, MFZ, MHF, A, CSP and MSH were responsible for data collection. MFZ perform laboratory tests, MSH, BL, MSA, NAR, LVS, AS and BA oversaw the study. MSH, AS and BL accessed and verified the data and did the data analysis. MSH, NAR, AK, BA, and BL wrote the first draft. MR, SD, RJC, FG, RNP, KL, HWU, LVS, and MSH review and editing the draft. All authors have read and approved the final version of the manuscript.

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Figures

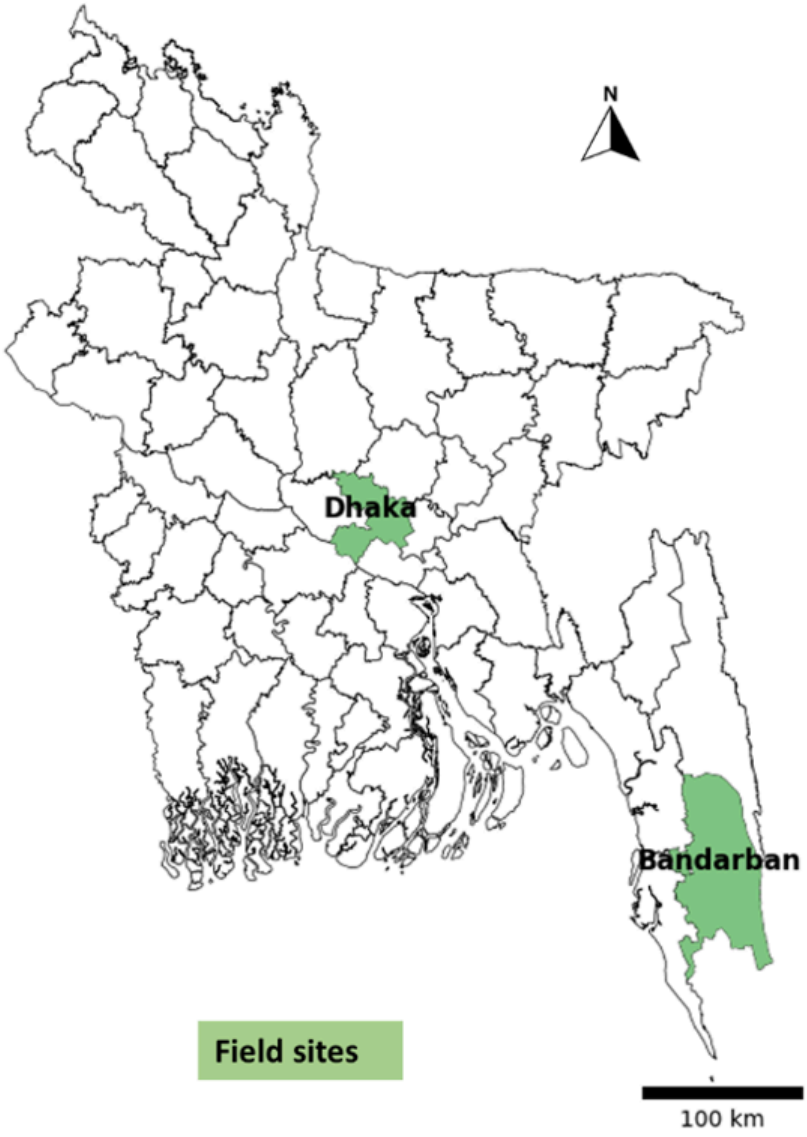


Figure 1

Study sites to evaluate a point-of-care G6PD testing service at the community level, 2025

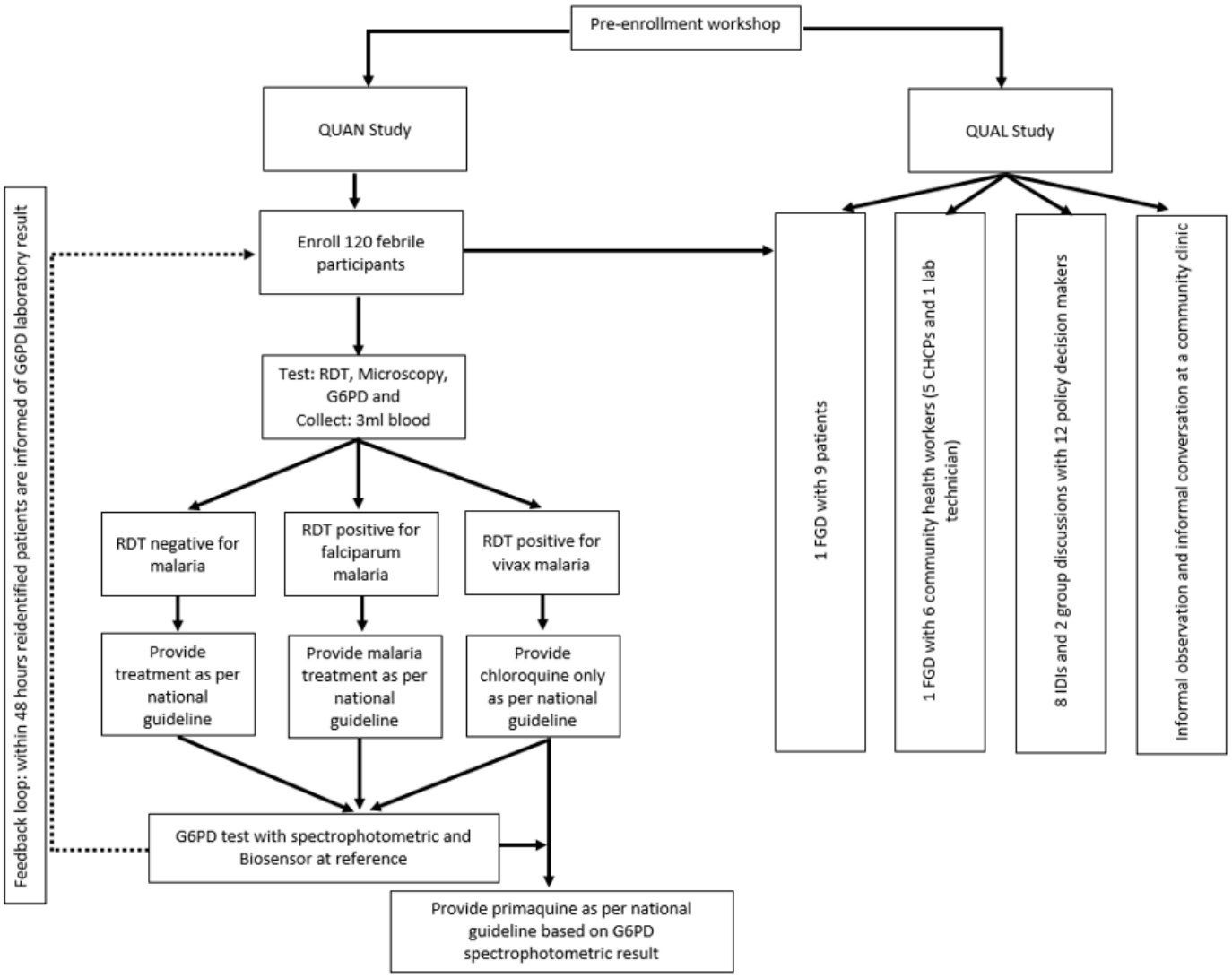


Figure 2

Schematic workflow of the QUAN and QUAL study.

QUAN: quantitative; QUAL: qualitative; RDT: rapid diagnostic test; G6PD: glucose-6-phopshate dehydrogenase; CHCPs: community health care providers; FGD: focus group discussions; IDIs: in-depth interviews

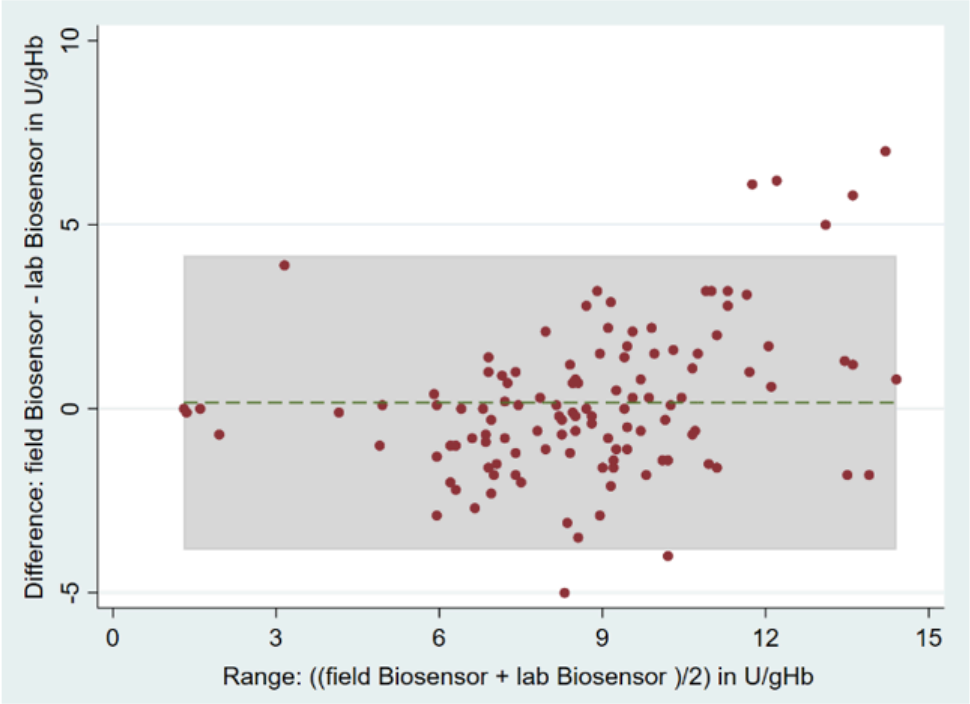


Figure 3

Comparison of the field Biosensor with reference laboratory Biosensor by Bland-Altman plot. Mean difference: 0.16 U/gHb, 95% LoA: -3.38 to 4.16 U/gHb (grey shaded area), green dashed line indicates mean difference.

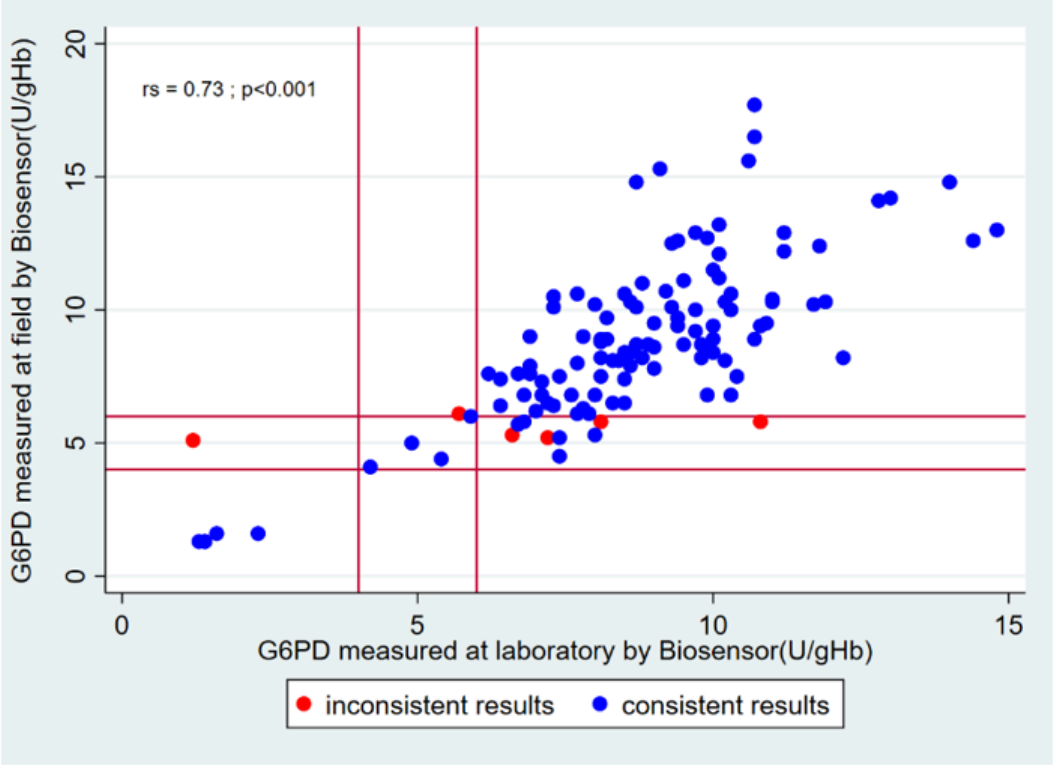


Figure 4

Scatter plot comparing G6DP results obtained from the field Biosensor with those from the reference laboratory Biosensor: $r_s = 0.73$ $p<0.001$, n = 119, red lines indicate 4U/gHb and 6U/gHb



Figure 5

Key factors affecting implementation of centralized G6PD testing

Supplementary Files

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- Annexure1.docx