

RESEARCH ARTICLE

# Use of the “STANDARD G6PD™” quantitative point-of-care test in neonates and infants

Gornpan Gornsawun<sup>1</sup>, Eh Moo<sup>1</sup>, Klay Htoo<sup>1</sup>, Supalak Chalermvisutkul<sup>1</sup>, Mary Ellen Gilder<sup>1,2</sup>, Phaw Khu Moo<sup>1</sup>, Laypaw Archusuksan<sup>1</sup>, Taco Jan Prins<sup>1</sup>, Borimas Hanboonkunupakarn<sup>3,4</sup>, Rose McGready<sup>1,2</sup>, Francois Nosten<sup>1,2</sup>, Germana Bancone<sup>1,2\*</sup>

**1** Shoklo Malaria Research Unit (SMRU), Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Mae Ramat, Tak, Thailand, **2** Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom, **3** Mahidol-Oxford Tropical Medicine Research Unit (MORU), Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, **4** Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

\* [germana@tropmedres.ac](mailto:germana@tropmedres.ac)



## OPEN ACCESS

**Citation:** Gornsawun G, Moo E, Htoo K, Chalermvisutkul S, Gilder ME, Moo PK, et al. (2026) Use of the “STANDARD G6PD™” quantitative point-of-care test in neonates and infants. PLoS One 21(7): e0346837. <https://doi.org/10.1371/journal.pone.0346837>

**Editor:** Samuel Kofi Tchum, Kintampo Health Research Centre, GHANA

**Received:** March 23, 2026

**Accepted:** June 22, 2026

**Published:** July 10, 2026

**Peer Review History:** PLOS recognizes the benefits of transparency in the peer review process; therefore, we enable the publication of all of the content of peer review and author responses alongside final, published articles. The editorial history of this article is available here: <https://doi.org/10.1371/journal.pone.0346837>

**Copyright:** © 2026 Gornsawun et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution,

## Abstract

Severe neonatal hyperbilirubinaemia represents a considerable cause of mortality and long term-morbidity in neonates born in low resource settings. Early identification of risk factors, such as glucose-6-phosphate dehydrogenase (G6PD) status, has the potential to prevent severe hyperbilirubinaemia and improve the clinical outcomes. The primary aim of the study was to assess equivalency of cord blood and neonatal capillary blood for diagnosis of G6PD deficiency using the quantitative point-of-care “STANDARD G6PD™” test (SD Biosensor, Korea). The secondary aim was to analyse changes in G6PD activity in the first 4 months of life. A total of 75 neonates born in Shoklo Malaria Research Unit (SMRU) clinics were selected based on their G6PD status assessed through routine cord blood screening using the “STANDARD G6PD™” test. Using activity thresholds established before in this setting, 25 G6PD deficient, 25 G6PD intermediate and 25 G6PD normal neonates were identified and re-tested using capillary blood collected within 24 hours of life and at day 7 by both “STANDARD G6PD™” test and gold standard spectrophotometric assay. They were also followed-up at 1 and 4 months of age to study haematologic and G6PD activity changes over time. The results showed that the “STANDARD G6PD™” can be used reliably up to one week of life for testing neonates using the same thresholds established in cord blood. Agreement of G6PD activity measured by the point-of-care test as compared to the gold standard spectrophotometry remained excellent at all sampling time-points. Nevertheless, G6PD activity assessed longitudinally in the same participants decreased over time, both at 1 month of age and at 4 months of age, and interpretation of results in female infants with intermediate activity might require different thresholds. The study demonstrated that the “STANDARD G6PD™” can

and reproduction in any medium, provided the original author and source are credited.

**Data availability statement:** De-identified participant data are available from the Mahidol Oxford Tropical Medicine Data Access Committee upon request from this link: <https://www.tropmedres.ac/units/moru-bangkok/bioethics-engagement/datasharing>.

**Funding:** The study was supported with fundings from SD Biosensor (Republic of Korea) to G.B. and Wellcome Trust (Grant 315982/Z/24/Z to F.N.). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. For the purpose of Open Access, the authors have applied a CC BY public copyright licence to any author accepted manuscript version arising from this submission.

**Competing interests:** The authors have declared that no competing interests exist.

effectively support clinical care in neonates and infants in populations with prevalent G6PD deficiency at the primary care level and especially in low-resource settings.

## Introduction

Bilirubin is a byproduct of haemoglobin catabolism. Levels of bilirubin are generally high in neonates (physiological hyperbilirubinaemia) due to increased destruction of fetal red blood cells and reduced capacity of immature enterohepatic systems to conjugate and excrete bilirubin. While physiological hyperbilirubinaemia is a normal finding in healthy neonates and generally does not require treatment, pathological neonatal hyperbilirubinaemia (NH) can lead to an accumulation of unconjugated bilirubin in the brain causing life-long neurological sequelae (kernicterus) and even death [1,2]. Glucose-6-phosphate dehydrogenase (G6PD) deficiency is one of the major risk factors for NH [3]. Phototherapy in equipped clinics is the mainstay of treatment but more severe NH might require exchange transfusion. Early diagnosis of abnormal G6PD phenotypes can help provide appropriate clinical care, both by avoiding treatment with possibly haemolytic drugs and also by extending observation and repeated bilirubin testing of neonates with this risk factor. Extended clinical care at birthing centers can dramatically improve outcomes in mobile and vulnerable populations with difficult access to care [4]. If not done at birth, diagnosis of G6PD status is often sought during the first week of life in neonates presenting with hyperbilirubinaemia and during the first month of life in neonates with prolonged jaundice.

Reference normal ranges of G6PD enzymatic activity have been established at the Shoklo Malaria Research Unit (SMRU) in cord blood samples collected at birth [5] and have been used since to identify neonates most at risk. Nevertheless, around 15% of neonates in this setting are not tested at birth using cord blood because they are born outside the clinic, and these neonates are tested at a later moment. It is important to confirm whether data obtained in cord blood are directly comparable to capillary whole blood collected within the first 24 hours of life or later. Published data indicate that there is a gradual decrease in enzymatic activity over time during the first 12 months of life [6–9] but there are no data on the changes in the first week and first month of life when the test is most often performed and large haematologic changes are observed. From the clinical point of view, it is important to understand whether G6PD activity thresholds established in cord blood can be used for the first week and month of life to diagnose neonates with abnormal G6PD levels.

The neonatal population in SMRU clinics shows high rates of NH (24% overall, [10]) which is multifactorial [11] and has strong genetic components [12]. In particular, G6PD deficiency which is very common among the Karen and Burman population attending SMRU clinics (15–20% in males, [13]), is associated with an increased risk to develop NH requiring phototherapy in neonates with deficient and intermediate G6PD levels [12]. The “STANDARD G6PD™” test (SD Biosensor, Korea; henceforth referred as Biosensor) is a quantitative enzymatic colorimetric assay that measures G6PD enzyme activity normalized by haemoglobin on 10 µl of capillary or venous blood. Results expressed in units per gram of haemoglobin (U/gHb) are provided

within two minutes on a portable, handheld analyzer and are used to classify individuals as G6PD normal, intermediate, or deficient according to established thresholds. This is currently the only commercially available quantitative point-of-care G6PD test and, in a previous evaluation study carried out here, it has shown to provide an accurate diagnosis of G6PD deficiency when used on cord blood at birth [5]. In SMRU, the device is routinely used to improve the clinical management of neonates at risk of NH and establishing its accuracy in capillary blood taken in the first month of life would extend these benefits to babies born outside SMRU clinics.

This study investigated whether levels of G6PD in cord blood and capillary neonatal blood are equivalent by Biosensor. Changes in G6PD activity in the first week and months of life in the same neonates followed-up prospectively until the 4-months were also analysed.

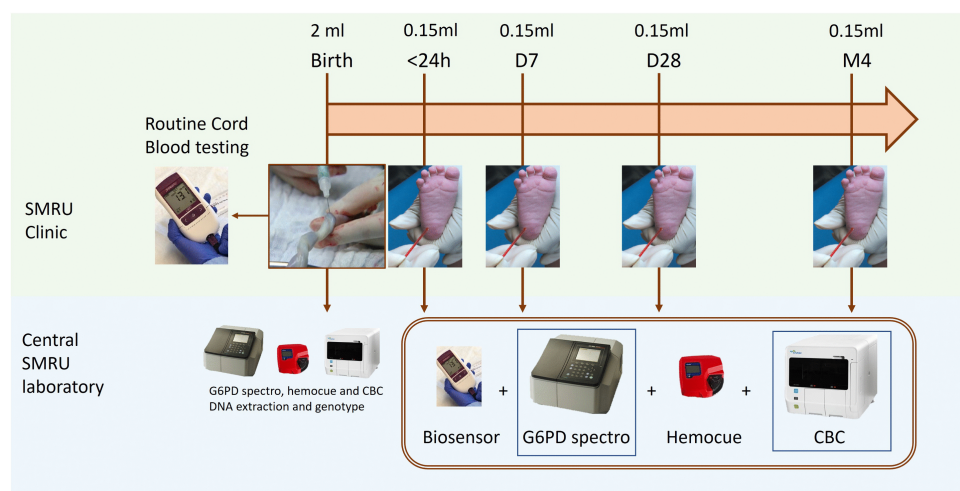
## Materials and methods

Pregnant women of Karen and Burman ethnicity attending Antenatal care (ANC) at SMRU Maw Ker Thai (MKT) clinic on the Thailand-Myanmar border were approached to participate in the study. In late pregnancy, eligibility (age 18 years or older with a singleton pregnancy) was assessed and written informed consent signed by the mother.

In SMRU clinics, cord blood samples are routinely collected in 2 ml EDTA tubes (after birth of the neonate and before delivery of the placenta) and analysed for G6PD activity using the Biosensor. The Biosensor test is performed by locally trained laboratory technicians following manufacturer's instructions. Two level quality control samples are analysed weekly. Neonates are classified based on thresholds established before [5] as follows: deficient ( $\leq 4.8$  IU/gHb), intermediate (4.9–9.9 IU/gHb, females only) and normal ( $\geq 4.9$  IU/gHb in males and  $\geq 10.0$  IU/gHb in females). Counselling and a multi-language red card indicating the baby G6PD status and substances to avoid are given to mothers of neonates with abnormal G6PD levels.

For the study, eligibility of neonates was assessed after results of G6PD were available from the site laboratory. Neonates were included if they were born at an estimated gestational age (EGA) by ultrasound  $\geq 35$  weeks with no severe maternal complications at birth and no severe neonatal illness, and had G6PD activity within the aforementioned thresholds.

The sampling plan is presented in Fig 1.



**Fig 1. Study sampling plan.**

<https://doi.org/10.1371/journal.pone.0346837.g001>

Cord blood samples of participating neonates were transported on ice packs to the central haematology laboratory of SMRU in Mae Ramat within 24 hours and further analysed by reference spectrophotometric assay (Pointe Scientific assay kit # G7583-180, lysis buffer # G7583-LysSB) on a SHIMADZU UV-1800 spectrophotometer (SHIMADZU, Japan) with temperature-controlled compartment. Samples were also analysed by complete blood count (CBC), including reticulocyte count, using a Sysmex XN-550 haematology analyzer (Sysmex Thailand Co., Ltd) and by Hemocue 301+ (HemoCue AB, based in Ängelholm, Sweden). Laboratory operators were blind to Biosensor clinic results.

At follow-up, a heel-prick sampling of 150uL of capillary blood on EDTA was carried out within 24 hours from birth, at day 7, at day 28 and at 4 months of life and transported to central haematology laboratory to be analysed by Biosensor, spectrophotometric assay, complete blood count, and Hemocue 301+. At the haematology laboratory, operators of Biosensor testing were blind to spectrophotometric results.

Genotyping for G6PD mutations following SMRU SOPs and protocol from Kim and colleagues [14] was performed from buffy coat obtained from cord blood. Genotype for Mahidol (487G>A), the most common G6PD mutation in this population, was performed first on all samples. Deficient and intermediate samples without Mahidol mutation, were then analysed for a panel of other common mutations [13]: Viangchan (871G>A), Chinese-4 (392G>T), Canton (1376G>T), Kaiping (1388G>A), Union (1360C>T), Mediterranean (563C>T). Full gene sequence was performed if none of these mutations were found using reference sequence ENSG00000160211 (transcript G6PD-006).

Routine clinical care was provided to all neonates included in the study. After birth, a routine pre-discharge total serum bilirubin test was carried out around 12–48 hours after birth, depending on clinical and risk factors. When neonates developed NH during the first week of life, they were admitted to the Special Care Baby Unit (SCBU) of SMRU and treated with phototherapy as per SMRU clinical guidelines. The gestational age at birth, assessed by early pregnancy ultrasound, were categorised as  $\leq 38$  and  $> 38$  weeks according to epidemiological studies conducted previously in the same population [11].

## Sample size and statistical analyses

The sample size to assess equivalence was calculated considering a Pearson's correlation ( $r$ ) between G6PD in cord blood and capillary blood of 0.95. Therefore, assuming a 95% confidence interval and acceptable half-width interval 0.05, the minimum sample size needed was 21; to account for 10% attrition, 25 neonates per class of phenotype (deficient, intermediate and normal) were included for a total of 75 samples.

G6PD status was defined using Biosensor and spectrophotometry thresholds established before in cord blood [5] as indicated in the methods. In order to understand whether universal thresholds could be used to define G6PD status at birth on cord blood and at follow-up visits, participants were classified as deficient, intermediate and normal based on Biosensor's thresholds used in routine clinical screening; the same classification was then applied to activity detected in the first capillary sample and at day 7. The same was done with data obtained using the gold standard assay, using spectrophotometry-defined thresholds. These analyses were carried out only on participants with samples collected at least at these 3 time points (CB, <24H and day 7).

Mean and SD or median and minimum-maximum values were reported for continuous variables. Correlation between activity in cord blood and at follow-up was assessed using Pearson's coefficient of correlation. Difference in G6PD activity and blood indices in paired samples were analysed by Wilcoxon Signed-Rank Test and paired T-test respectively.

Intraclass Correlation coefficient (ICC) and Bland-Altman plot were used to inspect correspondence between G6PD activity detected by Biosensor compared with the spectrophotometry assay. Bland-Altman analysis was performed by calculating the difference between individual paired measurements from the Biosensor and the gold standard. These differences were then plotted against the average of the two methods for each sample. The 95% Limits of Agreement were calculated as mean difference  $\pm 1.96$ SD.

Analyses were performed in SPSSv29 (IBM Corp., Armonk, USA).

## Ethics

The study protocol and its associated documents were approved by the Oxford Tropical Research Ethics Committee (OXTREC 530–23) and the Faculty of Tropical Medicine Ethics Committee (TMEC 23–023), Mahidol University. In addition, the Tak Province Border Community Ethics Advisory Board (T-CAB) was consulted for local feedback and advice.

## Results

### Study population

Study enrolment started on July 1<sup>st</sup> 2024 and was completed on April 20<sup>th</sup> 2025. Complete follow-up until 4 months of life for the last participant was reached on August 20<sup>th</sup> 2025. A total of 76 neonates were enrolled, with one excluded after the cord blood collection because the capillary sample collected within 24h of life could not be shipped to the central haematology laboratory for spectrophotometric analysis.

Neonates included in the study were 39 females and 36 males, with mean gestational age (min-max) of 39.1 (36.1–41.0) weeks. All the 75 participants were born by normal vaginal birth, had cord blood sampled, and had capillary blood collected within 24h after birth, at day 7, and at day 28. A capillary sample was taken at 4 months age for 95% (71/75) but one sample was clotted. Remaining participants were lost to follow-up.

Using biosensor to test cord blood samples, and applying activity thresholds used at the clinic, 26 neonates (3 females, 23 males) were deficient (activity  $\leq 4.8$  IU/gHb), 26 were female intermediate (activity between 4.9 and 9.9 IU/gHb) and 23 (10 females, 13 males) had normal G6PD status (activity  $\geq 4.9$  IU/gHb in males and  $\geq 10.0$  IU/gHb in females) at enrolment. Out of the 26 deficient samples, one sample (GCN-048) was later confirmed to be G6PD normal by spectrophotometric analysis on the same blood, on all subsequent blood samples (by both Biosensor and spectrophotometry) and by genotyping. Original logbook records and results recorded in the device were checked but no obvious explanation could be found for the incorrect result, suggesting a procedural error during testing or sample mis-identification. For this participant, results from cord blood sample only were excluded from analyses.

Using spectrophotometry to test cord blood samples and previously defined G6PD activity thresholds (100% normal activity = 13.3 IU/gHb; [5]), 25 neonates were identified as deficient ( $\leq 4.0$  IU/gHb), 20 as intermediate (4.1–9.3 IU/gHb) and 30 as G6PD normal ( $\geq 9.4$  IU/gHb) at enrolment.

The first capillary sample was collected at a median age (min-max) of 21 (12–25) hours (Figure S1 in [S1 File](#)). The second capillary sample was collected at a median age (min-max) of 7 (6–9) days of life. The third and fourth capillary samples were collected at a median age (min-max) of 28 (26–59) days of life and 122 (112–159) days of life respectively.

Overall results of G6PD activity according to phenotypic group using Biosensor are presented in [Table 1](#). Among all the 295 follow-up samples analysed by Biosensor, one sample collected within 24 hours of life did not provide a usable result by Biosensor (G6PD=NA with Hb=HI) and Hemocue; complete blood count in the sample showed Hb = 24.1 g/dL, the highest haemoglobin result recorded in the study.

Overall results of hemoglobin level by day of sampling by Biosensor, CBC and Hemocue are presented in Table S1 in [S1 File](#).

G6PD Mahidol was the prominent mutation found with 20 heterozygous females, 21 hemizygous males and 1 homozygous female. Fourteen females and 13 males were wild type (WT) and other mutations were found only in single subjects: Viangchan, Coimbra, Acores and a new mutation 50G>A on exon 2 (causing an amino acid change from Arginine to Glutamine in position 17) were found in heterozygous females, while Kaiping and Orissa were found in hemizygous males. [Table 2](#) reports the genotype distribution by G6PD phenotype as assessed by field Biosensor testing and laboratory-based spectrophotometry.

**Table 1. Median (minimum-max) G6PD activity by sampling day using the Biosensor (U/gHb) according to G6PD phenotype established at birth.**

|              |         | Cord blood | <H24       | D7         | D28        | M4        |
|--------------|---------|------------|------------|------------|------------|-----------|
| Deficient    | N       | 26         | 26         | 26         | 26         | 23        |
|              | Median  | 2.3        | 3.1 (135%) | 2.2 (96%)  | 1.6 (70%)  | 1.7 (74%) |
|              | Minimum | 1.2        | 1.7        | 1.7        | 1.0        | 1.3       |
|              | Maximum | 4.5        | 5.4        | 3.5        | 4.0        | 3.4       |
| Intermediate | N       | 26         | 25         | 26         | 26         | 24        |
|              | Median  | 7.5        | 8.8 (117%) | 7.2 (96%)  | 5.2 (69%)  | 5.0 (67%) |
|              | Minimum | 5.2        | 5.5        | 1.5        | 3.9        | 3.2       |
|              | Maximum | 9.8        | 13.5       | 12.1       | 11.1       | 8.9       |
| Normal       | N       | 22         | 23         | 23         | 23         | 23        |
|              | Median  | 12.5*      | 12.3 (98%) | 11.7 (94%) | 10.4 (83%) | 9.4 (75%) |
|              | Minimum | 10.7       | 10.1       | 10.2       | 8.2        | 5.7       |
|              | Maximum | 17.3       | 15.8       | 18.8       | 14.4       | 14.1      |

Percentage of activity in follow-up samples as compared to cord blood is reported in brackets.

<24H: sample collected within 24hours of life; D7: day 7; D28: day 28; M4: month 4.

\* Cord sample for GCN-048 was excluded.

<https://doi.org/10.1371/journal.pone.0346837.t001>

**Table 2. Genotype according to G6PD status in cord blood (by Biosensor and Spectrophotometry).**

| G6PD Status (CB)  | G6PD genotype   |              |    |
|-------------------|-----------------|--------------|----|
|                   | Hemi/homozygote | Heterozygote | WT |
| Biosensor         |                 |              |    |
| Deficient         | 24              | 2            | 0  |
| Intermediate      | 0               | 22           | 4  |
| Normal            | 0               | 0            | 23 |
| Spectrophotometry |                 |              |    |
| Deficient         | 24              | 1            | 0  |
| Intermediate      | 0               | 20           | 0  |
| Normal            | 0               | 3            | 27 |

<https://doi.org/10.1371/journal.pone.0346837.t002>

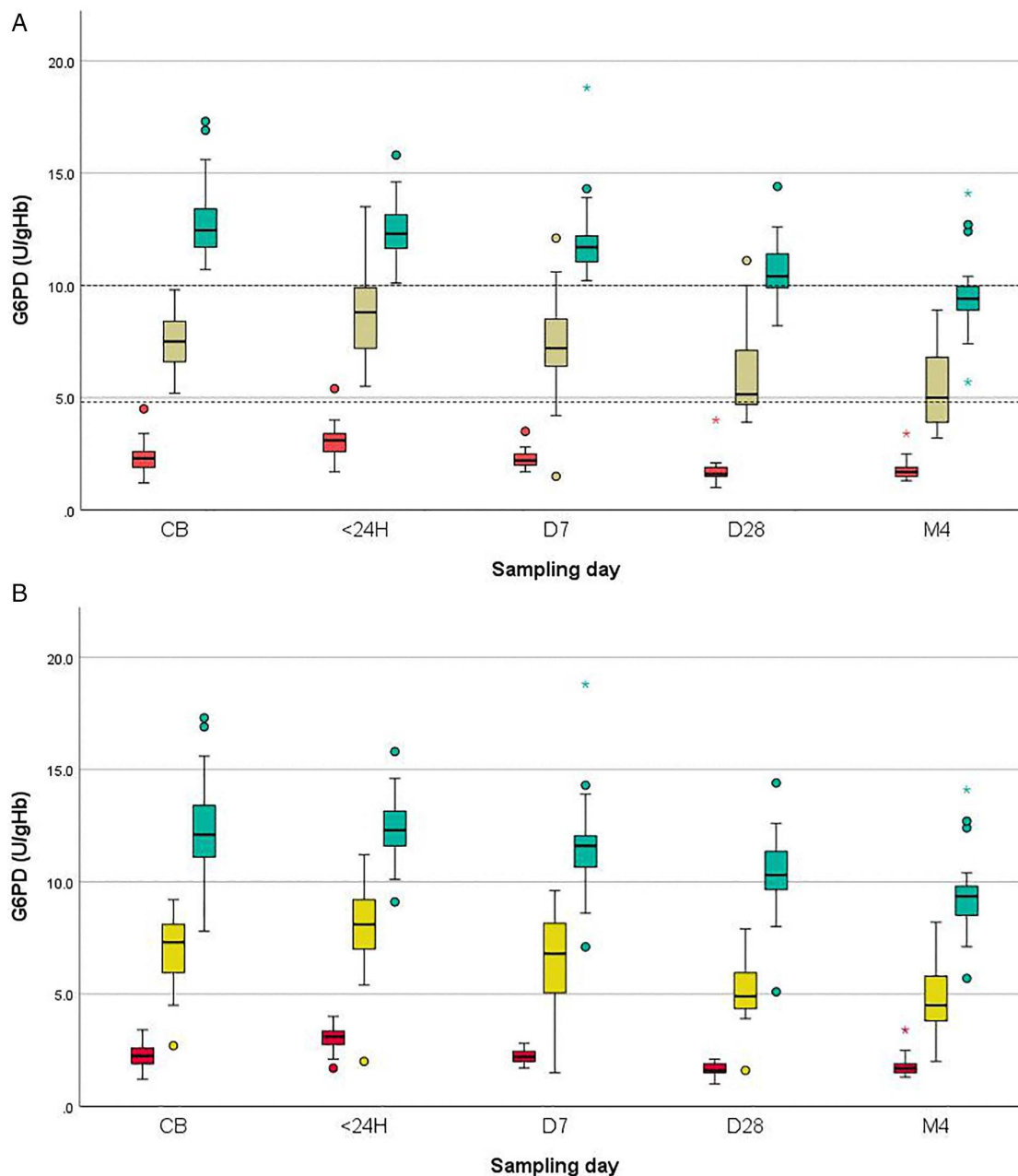
## G6PD activity by Biosensor: Comparison between cord blood and follow-up capillary samples

A graphic representation of individual G6PD activities over time is reported in Figure S2 in [S1 File](#).

Pearson's correlation coefficient between G6PD in cord blood and in capillary sample collected within 24h from birth was 0.937 ( $p < 0.001$ ; Figure S3 in [S1 File](#)). G6PD activity in paired samples was significantly higher at 24 hours compared with cord blood in capillary blood of deficient ( $p < 0.001$ ) and intermediate ( $p < 0.001$ ) participants. Activity was not different in G6PD normal participants ( $p = 0.685$ ; [Table 1](#)).

Pearson's correlation coefficient between G6PD in cord blood and day 7 was 0.945 ( $p < 0.001$ ) when analysed by Biosensor (Figure S4 in [S1 File](#)). G6PD activity in paired samples was significantly lower in normal capillary blood at day 7 compared with cord blood ( $p = 0.031$ ) but not in deficient ( $p = 0.625$ ) and intermediate ( $p = 0.494$ ) samples. Since activity was expected to decrease by 1 month of life [15] and after, no correlation analysis was performed for samples collected at day 28 and 4 months.

When comparing status classification, all G6PD deficient participants but one (a deficient heterozygote) would have been correctly identified as deficient by 24-hours and day-7 capillary blood using cord blood-derived thresholds



**Fig 2. (A) G6PD activity changes over time by Biosensor according to G6PD status established on cord blood.** Samples were classified as G6PD deficient (red bars), intermediate (beige bars) and normal (green bars). Dotted lines indicate activity thresholds. **(B) G6PD activity changes over time by Biosensor according to genotype.** Hemizygote or homozygote mutated genotype (red bars), heterozygote (yellow) and wild type (green). Outliers are indicated with circles and extreme outliers (>3 times the height of the box) with an asterisk. CB: cord blood; <24H: sample collected within 24hours of life; D7: day 7; D28: day 28; M4: month 4.

<https://doi.org/10.1371/journal.pone.0346837.g002>

(Fig 2A and Table 3, left pane). All G6PD mutated hemizygotes and homozygotes would have been correctly identified as deficient by 24-hours and day-7 capillary blood using cord blood thresholds (Fig 2B). Using the cord-blood derived thresholds for classification of activity in capillary blood collected at 24h would have resulted in misclassification as “normal” of 6

**Table 3. Comparison of G6PD status by Biosensor (using Biosensor CB thresholds).**

| G6PD status       | G6PD Status (<24H) |              |          | G6PD Status (D7) |              |          |
|-------------------|--------------------|--------------|----------|------------------|--------------|----------|
|                   | Deficient          | Intermediate | Normal   | Deficient        | Intermediate | Normal   |
| Cord blood        |                    |              |          |                  |              |          |
| Deficient (26)    | 25                 | <b>1</b>     | 0        | 26               | 0            | 0        |
| Intermediate (26) | 0                  | 19           | <b>7</b> | <b>3</b>         | 21           | <b>2</b> |
| Normal (22)       | 0                  | 0            | 22       | 0                | 0            | 22       |

Numbers in bold indicate misclassification of phenotype in the participant.

<https://doi.org/10.1371/journal.pone.0346837.t003>

participants with intermediate activity (in bold in Table 3, left pane). At day 7, there were 5 G6PD intermediate participants who would have been classified differently if applying cord blood-derived thresholds to activity detected in capillary blood (in bold in Table 3, right pane).

Comparison of G6PD activity between cord blood and follow-up capillary samples analysed by gold standard spectrophotometry is reported in S2 File.

### G6PD activity and haemoglobin by Biosensor compared to gold standard spectrophotometry and CBC

ICC for G6PD activity between methods overall and by sampling day was excellent (Table S2 in S1 File). Mean difference in G6PD activity by sampling day and overall was generally very small (Table 4) but limits of agreement were wide. Bland-Altman graphs for comparison of all data points and by day of sampling are presented in Fig 3 and Figure S6 in S1 File. Differences between methods were smallest at low activity levels and wider at higher activity levels.

For haemoglobin levels, overall ICC (95%CI) was 0.744 (0.17–0.97) between Biosensor and CBC and 0.86 (0.368–0.927) for Biosensor and Hemocue. Mean difference in haemoglobin between methods by sampling day and overall was generally acceptable (Table S3 and S4 in S1 File) with very wide limits of agreement. Bland-Altman graphs for comparison of all data points is presented in Figure S7 and S8 in S1 File for CBC and Hemocue respectively.

### Other haematologic changes between cord blood and follow-up samples

Together with expected decrease in enzymatic activity, changes in G6PD activity detected during the first four months of life might also be influenced by other physiologic modifications in the number of white and red blood cells, reticulocytes and in the concentration of haemoglobin (Table S5 and Figure S5 in S1 File). In particular, significant increase in WBC ( $p < 0.001$ ), RBC count ( $p < 0.001$ ) and reticulocyte percentage ( $p < 0.001$ ) at <24h of life in all G6PD phenotypic groups as compared to cord blood contributed to increased G6PD activity especially in G6PD abnormal participants.

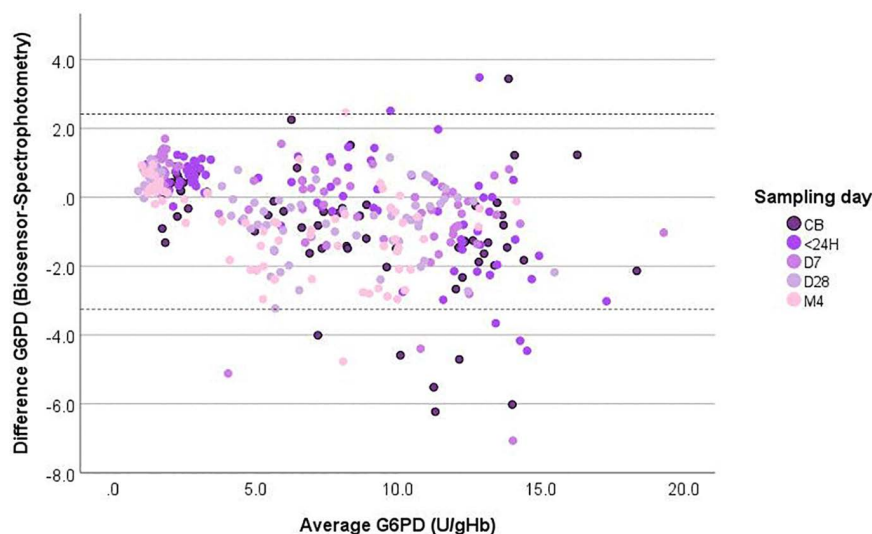
### G6PD and neonatal hyperbilirubinaemia

Among neonates born at >38 weeks of gestational age, a higher proportion of G6PD deficient and intermediate neonates needed phototherapy (8/22 and 1/24 respectively) as compared to G6PD normal (0/21). When G6PD activity was

**Table 4. Mean differences G6PD activity (Biosensor – Spectrophotometry) in U/gHb by sampling day.**

|      | CB            | H24           | D7            | D28           | M4            | Overall       |
|------|---------------|---------------|---------------|---------------|---------------|---------------|
| N    | 74            | 73            | 74            | 74            | 68            | 367           |
| Mean | –0.75         | –0.14         | –0.16         | –0.31         | –0.75         | –0.42         |
| SD   | 1.74          | 1.50          | 1.47          | 1.04          | 1.28          | 1.44          |
| LoA  | –4.16 to 2.66 | –3.08 to 2.79 | –3.05 to 2.73 | –2.34 to 1.72 | –3.26 to 1.76 | –3.25 to 2.41 |

<https://doi.org/10.1371/journal.pone.0346837.t004>



**Fig 3. Bland-Altman plot for G6PD activity (IU/gHb) in Biosensor and spectrophotometry for all data points.** Dotted lines represent limits of agreement.

<https://doi.org/10.1371/journal.pone.0346837.g003>

analysed by genotype, 7/20 hemi and homozygotes and 2/22 heterozygotes needed phototherapy as compared to 0/24 wild types.

## Discussion

This study demonstrated that G6PD activity measured from cord blood and capillary blood collected up to 7 days of life can be used with the same thresholds to identify, reliably, G6PD deficient neonates in this setting. The majority but not all of G6PD female neonates with intermediate activity could be identified using the same thresholds. Longitudinal analyses further confirmed the expected physiologic decline of G6PD activity during the first months of life across phenotypic and genotypic groups. The study also confirmed the good performance of the Biosensor compared to the gold standard spectrophotometry in neonates and infants up to 4 months of life.

Neonatal blood goes through extensive changes in the first weeks of life and this is the first study that analysed G6PD activity in cord blood and capillary blood collected up to 7 days of life. There are relatively little data describing longitudinal changes from venous cord and neonatal blood in term healthy babies; similar to what was observed here, blood indices tend to be higher in neonatal blood [16], particularly for WBC [17] due at least in part to physiological dehydration of infants fed on only colostrum on the first day of life (as recommended by the WHO and practiced at SMRU clinics). Together with the physiologic changes observed in G6PD activity, increased number of WBC and young reticulocytes would have impacted on total detected activity [18,19], especially in G6PD deficient and intermediate samples. However, only 1 out of 26 deficient neonates would be misclassified by the 24-hour Biosensor result and their classification as intermediate would have triggered increased clinical surveillance.

G6PD activity follows a clear bimodal distribution in males which allows a very good distinction between the normal and deficient phenotypes; the continuous distribution observed in females makes it more complicated to establish clear-cut thresholds and, consequently, performance of diagnostic tests (including the gold standard) at these thresholds are generally poorer in females. The current thresholds used in clinical practice are derived from physiologic and clinical observations with 30% activity as the “natural” threshold to define deficiency (corresponding to the flex in the male

bimodal distribution) and the 70% activity as the conventional (but arbitrary to some degree) threshold to identify “normality” in females. The limitations of establishing thresholds for a continuous variable are clear but so is the need to have a practical indication for use in clinical routine practice, especially at the point-of-care. The “STANDARD G6PD” Biosensor test has been validated before in older children and adults in the context of malaria testing and specifically in *P. vivax* endemic regions to support use of 8-aminoquinolines for radical cure. Diagnostic performance of thresholds indicated by manufacturers ( $\leq 4.0$  U/gHb for deficiency and  $> 6.0$  for normality in females) have been evaluated in several studies with excellent results [20–22]. It is acknowledged that both thresholds are conservative to maximise safety of 8-aminoquinolines administration.

Only 2 studies so far have analysed performance of the “STANDARD G6PD” test in neonates in cord blood or neonatal blood. The study from Manowong and colleagues [23] assessed G6PD activity in venous blood from 76 G6PD normal male neonates (aged 1–7 days) establishing a male median of 12.7 U/gHb with a proposed threshold to identify deficiency at 3.6 U/gHb; graphs of G6PD activity showed a clear bimodal distribution in males with a flex around 5 U/gHb. A second study [5], performed previously in this setting, detected a male median by Biosensor of 14.4 U/gHb and a threshold calculated to have the best sensitivity and specificity for genotypically hemi/homozygote participants at 4.8 U/gHb. Table S6 in S1 File shows data obtained before and data collected during this study; it includes a comparison with the manufacturer-defined thresholds established and validated in older children and adults in previous studies. Data obtained with the current study on a smaller sample size provided a median activity in G6PD wild type male and female participants of 12.1 U/gHb in cord blood.

Follow-up samples showed a similar median G6PD activity up to day 7 (95% of cord) with activity declining to around 80% at 1 month as compared to cord blood and around 75% at month 4. All hemizygous and homozygous deficient subjects up to 4 months of life would be identified correctly using the cord-blood threshold ( $\leq 4.8$  IU/gHb) or even the adult threshold ( $\leq 4$  U/gHb) with high sensitivity and specificity. On the other hand, identification of females with heterozygote genotype and deficient or intermediate activity using the cord-blood derived thresholds would have low to very low specificity after 7 days of life.

A study limitation was the length of follow-up of participants up to 4 months of life. As shown before [6], G6PD activity does not reach adult levels by 6 months of age and possibly not until one year of age. As a consequence, the Biosensor has not been evaluated yet in infants and children between 4 months and 2 years of age, leaving a diagnostic gap for safe administration of several drugs, including antimalarial radical cure in younger children. While using adult thresholds would probably identify most of the patients with lower activities, correct identification of females with the appropriate residual enzymatic activity to safely receive haemolytic 8-aminoquinolines might require assessment of age-specific thresholds.

In conclusion, this study has showed that the “STANDARD G6PD™” test can be used in both cord blood and capillary neonatal blood collected during the first week of life to provide reliable identification of G6PD abnormal neonates who are at increased risk of neonatal hyperbilirubinaemia. Equivalent performance in cord and capillary blood means enhanced use in different contexts and for different clinical needs. Additional evaluations outside Thailand (where consistent results have been obtained so far in neonates of Thai, Karen and Burman ethnicity) would further strengthen the generalizability of these findings and support wider use of this useful tool especially in low-resources settings.

## Supporting information

### S1 File. Tables and figures.

(DOCX)

### S2 File. G6PD activity by spectrophotometry: comparison between cord blood and follow-up capillary samples.

(DOCX)

## Acknowledgments

The authors wish to thank all the mothers for their collaboration and understanding; the study would not have been possible without the hard work and dedication of all SMRU staff involved.

## Author contributions

**Conceptualization:** Germana Bancone.

**Data curation:** Gornpan Gornsawun, Eh Moo, Klay Htoo, Supalak Chalermvisutkul, Mary Ellen Gilder, Phaw Khu Moo, Laypaw Archusuksan, Taco Jan Prins, Borimas Hanboonkunupakarn, Rose McGready, Francois Nosten.

**Formal analysis:** Mary Ellen Gilder, Rose McGready, Germana Bancone.

**Funding acquisition:** Germana Bancone.

**Investigation:** Gornpan Gornsawun, Eh Moo, Klay Htoo, Supalak Chalermvisutkul, Mary Ellen Gilder, Phaw Khu Moo, Laypaw Archusuksan, Taco Jan Prins, Borimas Hanboonkunupakarn, Rose McGready, Francois Nosten.

**Methodology:** Gornpan Gornsawun, Eh Moo, Klay Htoo, Supalak Chalermvisutkul, Mary Ellen Gilder, Phaw Khu Moo, Laypaw Archusuksan, Taco Jan Prins, Borimas Hanboonkunupakarn, Rose McGready, Francois Nosten, Germana Bancone.

**Project administration:** Germana Bancone.

**Supervision:** Francois Nosten.

**Validation:** Mary Ellen Gilder, Rose McGready.

**Writing – original draft:** Germana Bancone.

**Writing – review & editing:** Gornpan Gornsawun, Eh Moo, Klay Htoo, Supalak Chalermvisutkul, Mary Ellen Gilder, Phaw Khu Moo, Laypaw Archusuksan, Taco Jan Prins, Borimas Hanboonkunupakarn, Rose McGready, Francois Nosten.

## References

- Slusher TM, Zamora TG, Appiah D, Stanke JU, Strand MA, Lee BW, et al. Burden of severe neonatal jaundice: a systematic review and meta-analysis. *BMJ Paediatr Open*. 2017;1(1):e000105. <https://doi.org/10.1136/bmjpo-2017-000105> PMID: [29637134](#)
- Wouda EMN, Thielemans L, Darakamon MC, Nge AA, Say W, Khing S, et al. Extreme neonatal hyperbilirubinaemia in refugee and migrant populations: retrospective cohort. *BMJ Paediatr Open*. 2020;4(1):e000641. <https://doi.org/10.1136/bmjpo-2020-000641> PMID: [32537522](#)
- Kaplan M, Hammerman C. Glucose-6-phosphate dehydrogenase deficiency: a potential source of severe neonatal hyperbilirubinaemia and kernicterus. *Semin Neonatol*. 2002;7(2):121–8. <https://doi.org/10.1053/siny.2002.0099> PMID: [12208096](#)
- Olusanya BO, Osibanjo FB, Slusher TM. Risk factors for severe neonatal hyperbilirubinemia in low and middle-income countries: a systematic review and meta-analysis. *PLoS One*. 2015;10(2):e0117229. <https://doi.org/10.1371/journal.pone.0117229> PMID: [25675342](#)
- Bancone G, Gilder ME, Win E, Gornsawun G, Penpitchaporn P, Moo PK, et al. Technical evaluation and usability of a quantitative G6PD POC test in cord blood: a mixed-methods study in a low-resource setting. *BMJ Open*. 2022;12(12):e066529. <https://doi.org/10.1136/bmjopen-2022-066529> PMID: [36523222](#)
- Bancone G, Poe DD, Gornsawun G, Htway PP, Gilder ME, Archasuksan L, et al. Reference spectrophotometric values for glucose-6-phosphate dehydrogenase activity in two-to six-month-old infants on the Thailand-Myanmar border. *Wellcome Open Res*. 2024;7:273. <https://doi.org/10.12688/wellcomeopenres.18417.2> PMID: [38406309](#)
- Tang TK, Liu TH, Tang CJ, Tam KB. Glucose-6-phosphate dehydrogenase (G6PD) mutations associated with F8C/G6PD haplotypes in Chinese. *Blood*. 1995;85(12):3767–8. <https://doi.org/10.1182/blood.v85.12.3767.bloodjournal85123767> PMID: [7780161](#)
- Travis SF, Kumar SP, Paez PC, Delivoria-Papadopoulos M. Red cell metabolic alterations in postnatal life in term infants: glycolytic enzymes and glucose-6-phosphate dehydrogenase. *Pediatr Res*. 1980;14(12):1349–52. <https://doi.org/10.1203/00006450-198012000-00016> PMID: [6451861](#)
- Yang WC, Tai S, Hsu CL, Fu CM, Chou AK, Shao PL. Reference levels for glucose-6-phosphate dehydrogenase enzyme activity in infants 7-90 days old in Taiwan. *J Formos Med Assoc*. 2019.
- Turner C, Carrara V, Aye Mya Thein N, Chit Mo Mo Win N, Turner P, Bancone G, et al. Neonatal intensive care in a Karen refugee camp: a 4 year descriptive study. *PLoS One*. 2013;8(8):e72721. <https://doi.org/10.1371/journal.pone.0072721> PMID: [23991145](#)

11. Thielemans L, Peerawaranun P, Mukaka M, Paw MK, Wiladphaingern J, Landier J, et al. High levels of pathological jaundice in the first 24 hours and neonatal hyperbilirubinaemia in an epidemiological cohort study on the Thailand-Myanmar border. *PLoS One*. 2021;16(10):e0258127. <https://doi.org/10.1371/journal.pone.0258127> PMID: [34618852](https://pubmed.ncbi.nlm.nih.gov/34618852/)
12. Bancone G, Gornawun G, Peerawaranun P, Penpitchaporn P, Paw MK, Poe DD, et al. Contribution of genetic factors to high rates of neonatal hyperbilirubinaemia on the Thailand-Myanmar border. *PLOS Glob Public Health*. 2022;2(6):e0000475. <https://doi.org/10.1371/journal.pgph.0000475> PMID: [36962413](https://pubmed.ncbi.nlm.nih.gov/36962413/)
13. Bancone G, Chu CS, Somsakchaicharoen R, Chowwiwat N, Parker DM, Charunwatthana P, et al. Characterization of G6PD genotypes and phenotypes on the northwestern Thailand-Myanmar border. *PLoS One*. 2014;9(12):e116063. <https://doi.org/10.1371/journal.pone.0116063> PMID: [25536053](https://pubmed.ncbi.nlm.nih.gov/25536053/)
14. Kim S, Nguon C, Guillard B, Duong S, Chy S, Sum S, et al. Performance of the CareStart™ G6PD deficiency screening test, a point-of-care diagnostic for primaquine therapy screening. *PLoS One*. 2011;6(12):e28357. <https://doi.org/10.1371/journal.pone.0028357> PMID: [22164279](https://pubmed.ncbi.nlm.nih.gov/22164279/)
15. Thielemans L, Gornawun G, Hanboonkunupakarn B, Paw MK, Porn P, Moo PK, et al. Diagnostic performances of the fluorescent spot test for G6PD deficiency in newborns along the Thailand-Myanmar border: a cohort study. *Wellcome Open Res*. 2018;3:1. <https://doi.org/10.12688/wellcomeopenres.13373.1> PMID: [29552643](https://pubmed.ncbi.nlm.nih.gov/29552643/)
16. Greer R, Safarulla A, Koeppl R, Aslam M, Bany-Mohammed FM. Can fetal umbilical venous blood be a reliable source for admission complete blood count and culture in NICU patients? *Neonatology*. 2019;115(1):49–58. <https://doi.org/10.1159/000491993> PMID: [30300890](https://pubmed.ncbi.nlm.nih.gov/30300890/)
17. Scheffer-Mendoza S, Espinosa-Padilla SE, López-Herrera G, Mujica-Guzmán F, López-Padilla MG, Berrón-Ruiz L. Reference values of leukocyte and lymphocytes populations in umbilical cord and capillary blood in healthy Mexican newborns. *Allergol Immunopathol (Madr)*. 2020;48(3):295–305. <https://doi.org/10.1016/j.aller.2019.12.009> PMID: [32312563](https://pubmed.ncbi.nlm.nih.gov/32312563/)
18. Beutler E, Blume KG, Kaplan JC, Löhr GW, Ramot B, Valentine WN. International Committee for Standardization in Haematology: recommended methods for red-cell enzyme analysis. *Br J Haematol*. 1977;35(2):331–40. <https://doi.org/10.1111/j.1365-2141.1977.tb00589.x> PMID: [857853](https://pubmed.ncbi.nlm.nih.gov/857853/)
19. Bonsignore A, Fornaini G, Fantoni A, Leoncini G, Segni P. Relationship between age and enzymatic activities in human erythrocytes from normal and fava bean-sensitive subjects. *J Clin Invest*. 1964;43(5):834–42. <https://doi.org/10.1172/JCI104969> PMID: [14169512](https://pubmed.ncbi.nlm.nih.gov/14169512/)
20. Adissu W, Brito M, Garbin E, Macedo M, Monteiro W, Mukherjee SK, et al. Clinical performance validation of the STANDARD G6PD test: a multi-country pooled analysis. *PLoS Negl Trop Dis*. 2023;17(10):e0011652. <https://doi.org/10.1371/journal.pntd.0011652> PMID: [37824592](https://pubmed.ncbi.nlm.nih.gov/37824592/)
21. Martínez JC, Vélez-Marín V, Lopez-Perez M, Patiño-Lugo DF, Florez ID. Diagnostic accuracy of the point-of-care standard G6PD test™ (SD Biosensor) for glucose-6-phosphate dehydrogenase deficiency: a systematic review and meta-analysis. *Malar J*. 2024;23(1):327. <https://doi.org/10.1186/s12936-024-05144-1> PMID: [39488711](https://pubmed.ncbi.nlm.nih.gov/39488711/)
22. Zobrist S, Brito M, Garbin E, Monteiro WM, Clementino Freitas S, Macedo M, et al. Evaluation of a point-of-care diagnostic to identify glucose-6-phosphate dehydrogenase deficiency in Brazil. *PLoS Negl Trop Dis*. 2021;15(8):e0009649. <https://doi.org/10.1371/journal.pntd.0009649> PMID: [34383774](https://pubmed.ncbi.nlm.nih.gov/34383774/)
23. Manowong S, Tachawong N, Waiyo S, Charoenkwan P. G6PD enzyme activity in newborns and children: reference values by the quantitative colorimetric method and a comparison with the fluorescent spot test. *Biomed Sci Clin Med*. 2022;61(2):53–9.