Malaria Policy Advisory Group Meeting
23—24 March 2022, Geneva, Switzerland
Background document for Session 2

Technical consultation to review the classification of glucose-6-phosphate dehydrogenase (G6PD)

25 & 27 January 2022, virtual meeting

Summary

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is an X-linked genetic condition affecting an estimated 500 million people worldwide. It is a cause of neonatal jaundice, acute haemolytic anaemia, and chronic non-spherocytic haemolytic anaemia (CNSHA). The acute haemolysis can be triggered by eating fava beans ("favism"), exposure to several medicines, and infection. The occurrence of acute haemolytic anaemia after exposure to the 8-aminoquinolines tafenoquine and primaquine is an important concern, as these are the only available medicines that are effective against the hypnozoite stage of *Plasmodium vivax* and so they are needed for the elimination of this malaria parasite.

The first classification of G6PD-deficient variants was made in 1966 and updated by a World Health Organization (WHO) Working Group in 1985. This classification is still in use today. Since 1985, the full cDNA sequence of the G6PD enzyme has been published, enabling the full genetic characterization of variants. In the last 36 years, over 230 genetic variants have been identified. Many studies have reported a considerable overlap between Class II and Class III variants in terms of the severity of haemolysis and neonatal jaundice, raising questions about the value of having separate classes.

The WHO Global Malaria Programme convened a panel of temporary advisors in January 2022 to review the current classification and recommend changes where needed. WHO commissioned a literature review to examine the variability of G6PD activity for variants currently classified in Classes II and III, and also invited the presentation of an interim analysis of an individual patient meta-analysis investigating the variability of G6PD activity among genetic variants.

The literature review covered 59 studies published between 1966 and 2021. Data on 2255 hemizygous males with G6PD deficiency were included. The review identified 17 variants with 117 sets of genotypic/phenotypic information. Samples from 22 countries showed significant variability of residual enzyme activity for most genetic variants. Some variants showed activity values that were consistently >10% of normal (e.g. Orissa, Kalyan-Kerala), whereas others were consistently <10% (e.g. Coimbra, Union); however, most variants had values spanning the 10% threshold between Classes II and III. Some variants had relatively low variability between studies (e.g. Mediterranean), whereas others had high variability (e.g. A-$^{(202)}$). No variant in the study (except for Kalyan-Kerala) had a weighted mean activity value that was >30% of normal, but five variants had an activity range that crossed the 30% threshold. For Kalyan-Kerala, 55% of hemizygous males had activity that was >30% of normal, and for the other four variants (A-$^{(202)}$, Mahidol, Orissa, Seattle), 12–25% of individuals had activity that was >30%.

The interim analysis of the individual patient meta-analysis included studies from 2009 to 2021. It included 20 variants and phenotypic/genotypic data from 1118 individuals, including 336 hemizygous males. Of the data-rich variants, two showed limited variability in terms of enzyme activity (Mahidol:

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median 10.2%, range 0–32.5%; Viangchan: median 7.1%, range 0–17.5%). However, the A-$^{(202)}$ variant showed high variability (median 31.5%, range 1.7–154.1%), with 20% of individuals at >80% activity.

There was general consensus among the panellists that the variation in enzyme activity values for the same variant may reflect both technical and biological factors. The participants noted the shortage of reliable data (especially for some variants) and the need for more research on phenotypic/genotypic associations, using standardized methodologies and procedures across multiple populations to generate more reliable data on individual variants.

The panel concluded that:

- the variability of activity for most genetic variants across the arbitrary threshold of 10% that distinguishes between Class II and Class III variants presents a strong argument to abandon this separation in any future classification;
- Class I should be retained, as CNSHA is a rare chronic condition that is well characterized with specific clinical manifestations associated with G6PD deficiency;
- Class V was based on a single case reported in the literature but not confirmed by further studies and, therefore, does not need to be retained;
- because of the variability of activity for any single variant, the new classification needs to include a range around the reported median enzyme activity.

Details of the discussions and conclusions of the panel advisors are included in the main body of this report. The proposed revised classification and future research from the consultation are summarized in the box on the next page.

Revised classification

In future, G6PD variants should be classified based on the median residual enzyme activity expressed as a percentage of normal activity as follows:

WHO classification of G6PD variants in homozygous and hemizygous individuals		
Class	**Median of G6PD Activity**	**Haemolysis**
A	<20%	Chronic (CNSHA)
B	<45%	Acute, triggered
C	60–150%	No haemolysis
U	Any	Uncertain clinical significance

It should be emphasized that this system is for classifying genetic variants of G6PD and should not be used to classify individual patients with G6PD deficiency.

Currently, no variants have been identified in homozygous deficient females or hemizygous deficient males that have median G6PD enzyme activity falling between 45% and 60%. Therefore, a gap has been left between Classes B and C. If new variants are found with median G6PD enzyme activity in this range, these should be included in the "U" class and studied until solid evidence is found that they induce acute haemolytic anaemia (= Class B) or do not pose a haemolytic risk (= Class C). Based on new evidence, the thresholds may then need to be revisited.

Future research

WHO should consider developing standard criteria to characterize the genotypes and phenotypes of G6PD variants. This will also help to improve comparability across studies and inform the classification of new and existing variants. Any new variant should be assigned a tentative percent activity value only if this has been measured at steady state using a validated quantitative reference test in at least three samples from unrelated males. Other items to be considered include the number of individuals required to examine the distribution of G6PD activity, number of laboratory replica measurements, criteria to define normal reference values, genetic relationships among cases, methodologies for measuring G6PD activity, phenotypic screening and variant identification, and criteria for including or excluding subjects with concurrent infection or haemolysis. Many variants have now been identified at the molecular level for which important functional properties are unknown. It is desirable to measure at least K_m^{G6P} and thermostability for these and any new variants.

Future research should also aim at addressing important gaps in knowledge, namely the risk of severe haemolysis associated with known and potential triggers in already described variants and the identification of other biological factors that might influence haemolytic response (e.g. enzyme activity in reticulocytes).

Abbreviations

AHA	acute haemolytic anaemia
AMM	adjusted male median
CNSHA	chronic non-spherocytic haemolytic anaemia
G6PD	glucose-6-phosphate dehydrogenase
MPAG	Malaria Policy Advisory Group
NNJ	neonatal jaundice
PCR	polymerase chain reaction
WHO	World Health Organization

Background

Glucose-6-phosphate dehydrogenase (G6PD) deficiency

G6PD deficiency is an X-linked genetic condition affecting an estimated 500 million people worldwide. Most people affected live out their lives with no knowledge of their status, no symptoms and no complications. However, G6PD deficiency can lead to three clinical manifestations: neonatal jaundice (NNJ), acute haemolytic anaemia (AHA) and chronic non-spherocytic haemolytic anaemia (CNSHA).

In particular, AHA can be triggered by three possible causes, all linked to oxidative damage in red blood cells due to the reduced activity of the G6PD enzyme: eating fava beans ("favism"), drugs (such as 8-aminoquinolines like primaquine or tafenoquine), and infection.

The X-linked gene encoding G6PD is highly polymorphic, with over 230 variants identified at the molecular level *(1)*, many of which are polymorphic in different populations. The phenotypic expression in heterozygous females is highly variable, depending on the red cell mosaicism generated by the X-inactivation patterns. Therefore, in heterozygous females, the G6PD enzyme activity can vary between normal and that of a G6PD hemizygous male.

G6PD deficiency is more common in malaria-endemic countries. There is evidence that the heterozygous state (females) confers protection from severe infection by *Plasmodium falciparum* and possibly *P. vivax*. All G6PD-deficient variants entail haemolytic risk, but the range of severity of haemolysis differs for each variant.

Current World Health Organization (WHO) classification and guidance

The first international WHO meeting on G6PD was convened in December 1966, when just 20 G6PD variants had been described according to their biochemical characteristics, such as percent activity (measured by gold standard spectrophotometric assay), electrophoretic mobility (K_m) value, activity on substrate analogues, pH optimum, and thermostability *(2)*. This meeting proposed that an indication be given for each variant in terms of the enzyme activity in males. This led to a proposed classification published by Yoshida et al. *(3)*. WHO convened a Working Group on G6PD in 1985, which made some minor modifications to the Yoshida classification *(4)*. This modified classification remains in use today.

G6PD classification	Level of residual enzyme activity (% of normal)
Class I (Severe enzyme deficiency with CNSHA)	<10% with CNSHA
Class II (Severe)	<10%
Class III (Moderate to mild)	10–60%
Class IV (Very mild or no enzyme deficiency)	60–150%
Class V (Increased enzyme activity)	more than twice normal

Recent developments

Since the publication of the WHO classification in 1985, the full G6PD cDNA sequence has been published *(5).* This has enabled the identification of variants by their genotype, rather than relying on the biochemically measured level of G6PD activity. In the last 36 years, over 230 variants have been identified at the molecular level *(1)*.

Since 1985, and even before, several drugs have been shown to cause haemolytic anaemia in G6PD-deficient patients. The most notable have been antimalarials including chlorproguanil-dapsone, primaquine and tafenoquine. These drugs have been shown to trigger potentially life-threatening haemolysis in patients with Class II and Class III variants. From a public health point-of-view, the risk of haemolytic anaemia with the 8-aminoquinolines (primaquine and tafenoquine) is of particular concern, as these are the only drugs currently available that are active against the hypnozoite stage of *P. vivax* and so they are needed for the elimination of this malaria parasite.

Many studies have reported that there is considerable overlap between Class II and Class III variants in terms of the severity of haemolysis and NNJ, raising questions about the value of having separate classes.

The cut-off point between Class III and Class IV has also been questioned in relation to the threshold for "normal" G6PD activity, which was originally set at >60%. This has subsequently been set at >70% for clinical trials with tafenoquine *(6)*. A 2014 WHO consultation on point-of-care G6PD tests *(7)* recommended a threshold of G6PD activity >80% in heterozygous females and >30% in hemizygous males in order to minimize the haemolytic risks related to primaquine anti-relapse therapy *(8)*.

Given the time since the G6PD classification was established and the developments in the interim, the WHO Genomics Initiative identified the revision of the current classification scheme as a priority. It recommended that the WHO Global Malaria Programme convene a Technical Consultation to review and propose a revision of the classification in light of all the information and data currently available. This was endorsed by the WHO Malaria Policy Advisory Group (MPAG; formerly the Malaria Policy Advisory Committee [MPAC]) in October 2019.

Objectives

1. Review the results of literature searches commissioned by the WHO Global Malaria Programme and academic institutions in order to assess the variability of enzyme activity for the main G6PD genetic variants of public health interest.

On the basis of these study findings:

2. Review the distribution of G6PD activity in relation to the threshold of enzyme activity adopted to define severe G6PD deficiency.

3. Review the distribution of G6PD activity in subjects with a deficiency in relation to detection levels for current qualitative and semiquantitative point-of-care G6PD tests.

The results of this Technical Consultation are expected to be relevant to work on establishing policy and product specifications for point-of-care G6PD tests, and for the use of 8-aminoquinolines for the radical cure of *P. vivax*.

Process

1. The WHO Global Malaria Programme commissioned a literature review to gather information on the mean and variability of G6PD activity for variants currently classified in Classes II and III *(9)*, and also invited a presentation of the interim analysis of a systematic review and meta-analysis investigating the variability of G6PD activity among genetic variants *(10)*.

2. A panel of WHO temporary advisors (acting in their personal capacity) was convened remotely for two half-day virtual sessions on 25 and 27 January 2022 (see Annex 1, list of pre-reads; Annex 2, list of participants; and Annex 3, agenda of the meeting).

3. The panel of advisors reviewed the results of the two literature reviews and the implications for the classification of G6PD variants. At the end of the meeting, they held a closed session[1] during which they agreed on the conclusions of the meeting.

4. The report of the meeting was prepared by Ian Boulton (rapporteur) and shared with all participants for comment. Their inputs were then taken into account in preparing the final report for presentation to MPAG.

Report of the Technical Consultation

Issues with current WHO classification

The panel of advisors identified several problems with the existing classification:

1. The intended use of the classification of variants published in 1986 was to group genetic variants according to the mean or median levels of G6PD biochemical activity. However, this classification has been used to assign patients to the different levels of severity of G6PD deficiency based on measured enzyme activity.

2. It has become clear that there is significant overlap in the clinical manifestations of several G6PD variants in Classes II and III.

Literature review findings

Nannelli et al. *(9)*

The literature review screened 2200 unique articles by title and abstract, and identified 393 full-text records for assessment. Applying strict eligibility criteria[2], the review identified 59 studies published from 1966 to 2021 that used biochemical criteria, DNA analyses, or both. Data were gathered from 2255 hemizygous males with G6PD deficiency. Based on the available data, 17 variants and 117 sets of genotypic/phenotypic associations were included in the analysis. The variants included were:

[1] Dr Mary Relling and Professor Benedikt Ley did not attend the closed session.

[2] The study included data that could be extracted on all of the following: a) the G6PD variant was clearly identified by biochemical and/or molecular analysis; b) data were available for males ; c) G6PD activity was expressed in absolute units or as a percentage of normal G6PD values obtained in the same laboratory; d) an appropriate quantitative method was used to measure G6PD activity; e) variant activity was reported for at least three individuals (except for variants with less than three eligible studies with at least three individuals, for which articles with fewer than three individuals were also included in the analysis); and f) measurement of G6PD activity was made in steady state (not in the haemolytic or post-haemolytic period).

A-(202), A-(968), Aures, Cairo, Canton, Chatham, Coimbra, Cosenza, Kaiping, Kalyan-Kerala, Mahidol, Mediterranean, Orissa, Seattle, Union, Vanua Lava and Viangchan.

The geographical distribution of samples included is shown in Table 1.

Table 1. Geographical distribution of samples identified in Nannelli et al. *(9)*

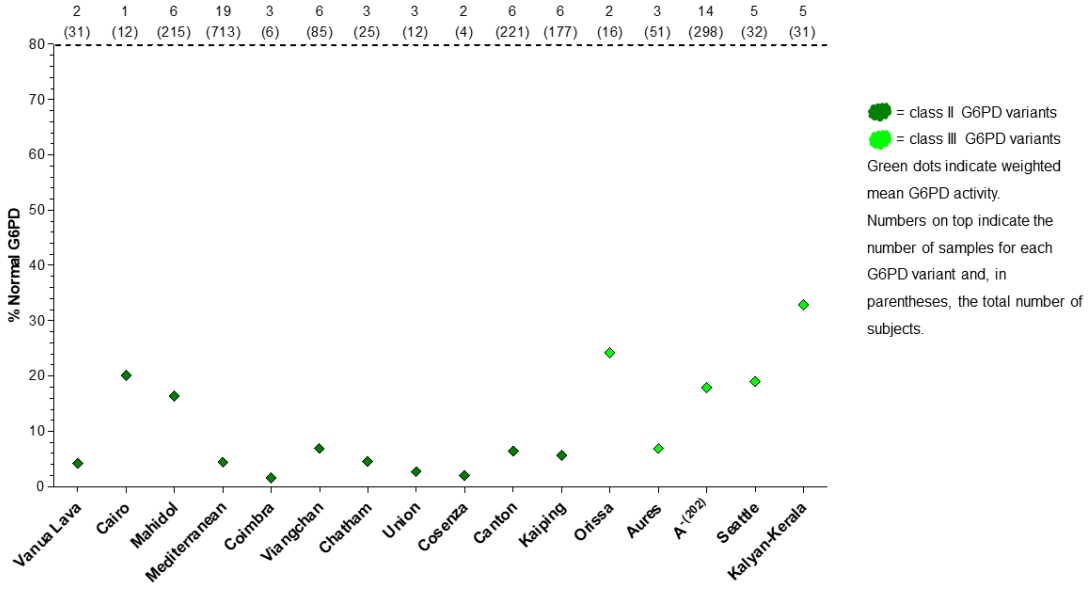
Geographical origin	Orissa	Aures	A-(202)	Vanua Lava	Cairo	Mahidol	Mediterranean	Coimbra	Seattle	Viangchan	Kalyan-Kerala	A-(968)	Chatham	Union	Cosenza	Canton	Kaiping	Total samples	(%)
Algeria			3				1		1									6	(5.1)
Bangladesh	1					1					1							3	(2.6)
Brazil			1															1	(0.9)
Cambodia										1								1	(0.9)
China										1				1		3	10	15	(12.8)
France							1											1	(0.9)
Greece							1		1									2	(1.7)
India	2						1				4							7	(6.0)
Indonesia				2				1		2			1					6	(5.1)
Iraq							3						2					5	(4.3)
Italy			2				17	1	8					1	2			31	(26.5)
Mozambique			1															1	(0.9)
Myanmar						1												1	(0.9)
Nigeria			3															3	(2.6)
Occupied Palestinian territory, including east Jerusalem			1		1		1											3	(2.6)
Portugal			1					1	1			1						4	(3.4)
Saudi Arabia		1	1				2											4	(3.4)
Sudan			1															1	(0.9)
Thailand		2				2				2					2	2	2	12	(10.3)
Thai-Myanmar						2										1		3	(2.6)
Tunisia			1				1											2	(1.7)
USA			4				1											5	(4.3)
Total	3	4	19	2	1	6	29	3	11	6	5	1	3	2	4	6	12	117	(100)

The subjects included in the review were recruited from 22 countries. The majority of the data were related to four variants (A-(202), Mediterranean, Seattle and Kaiping) and were from three countries (China, Italy and Thailand).

Analysis of the distribution of mean/median G6PD activity reported in each study showed that the Orissa and Kalyan-Kerala variants consistently have activity values above 10% of normal (the current threshold between Class II and Class III variants). Coimbra, Chatham, Union and Cosenza variants have values consistently below 10%. However, all the other variants (A-(202), Canton, Kaiping, Mahidol, Mediterranean, Seattle, Viangchan) have mean/median values that span the 10% threshold. For some variants (e.g. Mediterranean), the results are quite tightly grouped, but for others (e.g. A-(202)), there is a much greater degree of variability. Data from studies that identified variants based on biochemical criteria alone aligned well with data from studies using DNA-based identification.

The weighted mean activity for 16 variants is shown in Fig. 1. The A-$^{(968)}$ variant was excluded, as there was only one value available.

Fig. 1. Weighted mean G6PD activity for variants identified in Nannelli et al. *(9)*



With the exception of the Kalyan-Kerala variant, the weighted average activity for all other variants is below 30%. The Cairo and Mahidol variants (Class II) have a weighted average activity above 10%, and the Aures variant (Class III) falls below 10%.

Sample variability was estimated by pooling data from all samples available for each variant and including information about the variability observed in each sample.

Analysis of the estimated overall variability showed that, for 12 variants, values for a significant number of samples span the 10% cut-off. This is observed for the following variants: A-$^{(202)}$, Aures, Canton, Chatham, Kaiping. Kalyan-Kerala, Mahidol, Mediterranean, Orissa, Seattle, Vanua Lava and Viangchan. For five variants, values overlap the 30% cut-off. In particular, for the Kalyan-Kerala variant, an estimated 55% of samples have activity values above the 30% cut-off. Considering the spread around the median (assuming a normal distribution of results), four variants (A-, Mahidol, Orissa and Seattle) have an estimated 12–25% of males with G6PD activity above 30%. The authors concluded that, with the exception of the Kalyan-Kerala variant, all variants falling into Classes II and III have a median enzyme activity below 30% of normal.

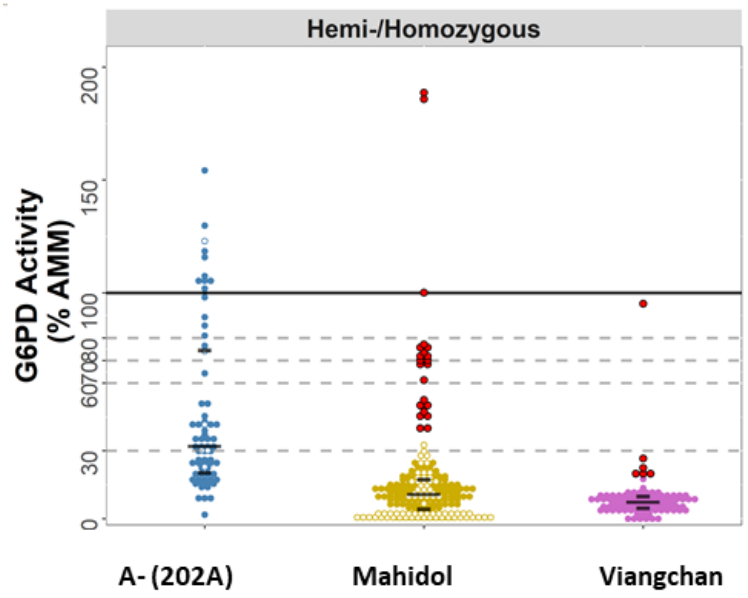
The variability among samples may be due to methodological as well as biological factors. The methods used in the studies conducted over a 55-year period were not always well described and documented in the publications. Results may have been influenced by variability in spectrophotometric methods, procedures for specimen storage and transport, blood sample preparation, reaction mixture and temperature control in assay procedures, use of single or replicate tests, and the way white cells were or were not removed. However, these concerns were not considered great enough to significantly distort the overall results.

Pfeffer et al. *(10)*

This is an *interim* analysis of a larger meta-analysis to assess the range of G6PD activity for known G6PD genotypes.[3] A literature review screened 838 unique articles by title and abstract, and identified 153 full-text records for assessment. A set of strict eligibility criteria were applied to generate a preliminary dataset for the purposes of this review. This yielded 13 datasets published since 2005 using one of three common spectrophotometry assay kits for individual patient data analysis. All studies were conducted between 2009 and 2021, and variants were identified by DNA analysis. The database contained phenotypic/genotypic data from 1118 G6PD-deficient individuals, of which 336 were hemizygous males, for 20 different variants (three data-rich [n≥30] and 17 data-poor). Data were generated in eight countries, the majority being from South-East Asia (77%), followed by Africa (14%) and the United States of America (8%). Some of the datasets used were also included in Nannelli et al.'s *(9)* literature review.

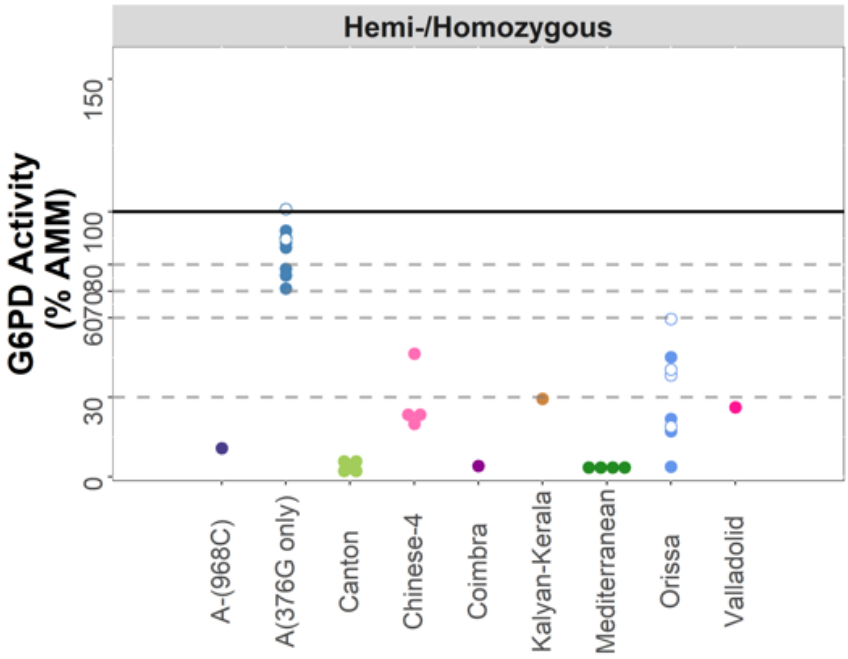
The G6PD activity values for data-rich variants (A-$^{(202)}$, Mahidol, and Viangchan) among male hemizygous and female homozygous individuals are shown in Fig. 2. To mitigate the influence of extreme measurements, outliers were defined for all data-rich variants and excluded from analyses. The data on homozygous/hemizygous individuals show a much wider variation for the A-$^{(202)}$ variant (median = 31.5%, range 1.7–154.1%) than for the other two variants, Mahidol (median = 10.2%, range 0–32.5%) and Viangchan (median = 7.1%, range 0–17.5%). Fig. 3 shows the data for the data-poor variants among male hemizygous and female homozygous individuals.

Fig. 2. G6PD activity levels for data-rich variants among male hemizygous and female homozygous individuals, Pfeffer et al. *(10)*



[3] The full analysis is expected to be completed by March 2022.

Fig. 3. G6PD activity levels for data-poor variants among male hemizygous and female homozygous individuals, Pfeffer et al. *(10)*



Note. G6PD activity (% AMM) as measured by spectrophotometry among 93 individuals confirmed by PCR or sequencing to carry a genetic variant other than A-, Mahidol, Viangchan. Individuals <1 year of age or positive for malaria were excluded. Horizontal lines indicate diagnostic thresholds: 100% (black), 80%, 70%, 60% and 30% (grey, dashed) G6PD activity. Homozygotes are indicated using hollow points.

For the data-rich variants, observations were binned according to the commonly used diagnostic thresholds for G6PD deficiency: severe (<30%) or intermediate (<60%, <70% or <80%). These results are shown in Table 2.

Table 2. Number and percentage of individuals falling into the various diagnostic categories for data-rich variants, Pfeffer et al. *(10)*

Variant	n	Studies (n)	Number (%) included using diagnostic thresholds				
			<30%	<60%	<70%	<80%	≥80%
A-(202A)							
Hemi-/Homozygous	72	5	33 (45.8)	55 (76.4)	56 (77.8)	58 (80.6)	14 (19.4)
Mahidol							
Hemi-/Homozygous	201	5	200 (99.5)	201 (100)	201 (100)	201 (100)	0 (0)
Viangchan							
Hemi-/Homozygous	90	3	90 (100)	90 (100)	90 (100)	90 (100)	0 (0)

Virtually all Mahidol and Viangchan variants fall below the 30% threshold in hemizygous and homozygous individuals. However, in almost 20% of cases, the A-$^{(202)}$ variants show >80% of normal activity in homozygous/hemizygous individuals.

Some of the variation has been attributed to imprecise assay techniques. Furthermore, a limitation of the survey is that, in 10 out of 13 studies, participants were only genotyped if they met pre-defined G6PD activity thresholds used in phenotypic screening tests. Therefore, the results may have been skewed towards the lower end of the activity spectrum. However, the authors argued that the overall variability observed was too great to be explained solely by confounding factors.

Discussion on the literature reviews

The panel appreciated the amount and quality of work reflected in these two reviews. The inclusion of both individual and sample-level data was particularly appreciated.

The two reports covered largely overlapping sets of variants. For most variants, there was generally good agreement between results, except for the A-$^{(202)}$ variant. The higher median value derived by Pfeffer et al. *(10)* for G6PD A- might be attributable to the inclusion of subjects with higher G6PD activity due to unidentified methodological or biological factors.

These reviews showed that rich datasets are available for six G6PD variants: A-$^{(202)}$, Canton, Kaiping, Mahidol, Mediterranean and Viangchan. The reviews demonstrated that there is still a shortage of reliable published data on the activity of different variants, and there is a need for more widespread genotyping of variants from populations in different geographical areas.

It was noted that the variation in phenotypic screening before genetic identification of G6PD variants was not always documented in publications. In Nannelli et al. *(9)*, 33 out of 117 sets had G6PD phenotypic screening, while in Pfeffer et al. *(10)*, 10 out of 13 studies had G6PD phenotypic screening; however, the panel did not think that this invalidated the overall findings from the two reviews.

The literature reviews revealed differences in the methods used to obtain the published results and the quality or reproducibility of the spectrophotometry methods. It was noted, however, that the individual patient data analysis by Pfeffer et al. *(10)* showed considerable variability for the A-$^{(202)}$ variant and several “outliers” for the Mahidol and Viangchan variants, despite 75% of the spectrophotometry data being from the same Trinity Biotech spectrophotometry assay.

The measurement of G6PD activity in blood samples could be affected by the time taken and temperature during transportation of the samples to the laboratories in less-than-ideal storage conditions, as well as by the specific technique used to remove the white blood cells to prepare the blood samples. These important details were rarely provided in the publications.

There was general consensus that variation in enzyme activity values for the same variant may reflect both technical and biological factors. The panel supported work on developing more standardized research methods to study G6PD genotypic/phenotypic association in order to increase the availability of reliable data on individual variants.

Nannelli et al.’s *(9)* review of the published literature assumed that enzyme activity measurements of male hemizygous individuals were normally distributed (based on the limited individual data available), but this should be confirmed by analysing additional individual data using standardized approaches.

It would be highly desirable to assign median enzyme activity values to each variant as part of characterizing them within a classification system, but it was felt that this is not yet possible for many variants.

In future, it would be ideal for G6PD surveys to include the variant's genotype, phenotypic presentation, and clinical risk in different populations and geographical areas. This will be more feasible as genetic methods become more widely available and less expensive. Studies based on G6PD phenotypic screening should also genotype a sample of participants considered to be normal in order to identify possible genetic variants undetected by the screening assays (that can only discriminate between <30% and >30%). This will enable detection of individuals who carry a G6PD mutation but present with less severe enzyme activity deficiency.

Discussion on revision of the G6PD classification

The panel recommended that any revision of the classification system be as clear and simple as possible. It should be practical and relevant for clinical use, including in challenging field conditions where access to genotyping may not be easy or the volume of patients makes routine genotyping impractical.

There was concern that the current G6PD classification *(4)* has been often used as a way to classify patients based on enzyme activity, rather than a way to classify individual variants and their intrinsic potential haemolytic risk to patients. This needs to be made very clear in any update to the current classification.

The panel agreed that, in view of the significant overlap in the distribution of activity among variants allocated to Class II and Class III, the distinction based on the 10% threshold is no longer useful. The panel agreed that these two classes could be merged into one, with no distinction between "severe" and "moderate to mild" deficiency.

Class I variants manifest with severe G6PD deficiency and CNSHA, which is a rare congenital condition. Therefore, it was agreed that this class should be retained. There has been only one example of a Class V variant (activity >150%) – G6PD Hektoen – and there has been no other since. It was agreed that there was no practical purpose in retaining Class V.

The classification of variants based on mean or median residual enzyme activity serves a clear purpose in identifying variants that may cause haemolysis. However, the panel also discussed the fact that there is limited data on haemolytic response in many variants and that the same variant may cause different levels of haemolysis in different patients. A variant such as Kalyan-Kerala, which is the second most common variant in India, has a median enzyme activity of 32.2%. While this may mean that there is enough G6PD activity to prevent serious haemolytic episodes in some male hemizygous patients, there could be serious haemolysis in other subjects. In practice, any classification should include a range around the median G6PD activity to reflect this variability.

While some studies have used thresholds of 70% enzyme activity as exclusion criteria in drug development studies, it was felt that it would be too disruptive to other work to raise the threshold for normal hemizygous males above 30% of normal activity in a revised classification of variants. There was also no clear consensus about whether 70% or 80% would be a more appropriate value for a threshold of G6PD enzyme activity in female heterozygous individuals.

Conclusions from the consultation

Revised classification

In future, G6PD variants should be classified based on the median residual enzyme activity in male hemizygous individuals for each variant expressed as percentage of normal activity as follows:

WHO classification of G6PD variants in homozygous and hemizygous individuals		
Class	**Median of G6PD Activity**	**Haemolysis**
A	<20%	Chronic (CNSHA)
B	<45%	Acute, triggered
C	60–150%	No haemolysis
U	Any	Uncertain clinical significance

It should be made clear in all publications that this system is strictly for classifying genetic variants of G6PD and applies primarily to hemi/homozygous individuals carrying a particular mutation. It should not be used to classify individual patients.

The above G6PD classification scheme is binary, and each genetic variant is defined as deficient or normal under this classification. Class B indicates G6PD deficiency without CNSHA. Class U, regarded as of ”uncertain clinical significance”, will serve as a temporary classification for newly discovered G6PD variants until the residual activity can be reliably measured in at least three samples from unrelated males in a steady state and the clinical significance is assessed.

By reviewing/using the combined data from the reviews by Nannelli et al. *(9)* and Pfeffer et al. *(10)*, each variant can be tentatively assigned a percent activity value. These values will be subject to review as new data become available. The percent activity value should be calculated from the median value of genotypically normal male individuals, not from the AMM, considering the overlap of most variants across the 10% threshold.

Currently, no variants have been identified that have median G6PD enzyme activity values in male hemizygous and/or female homozygous individuals falling between 45% and 60%. Therefore, a gap has been left between Classes B and C. If new variants are found with median G6PD enzyme activity between 45% and 60%, these should be included in the “U” class and studied until solid evidence is found that they induce AHA in male hemizygous and/or female homozygous individuals (= Class B) or pose no haemolytic risk (= Class C). The thresholds will then need to be revisited as new evidence becomes available.

Future research

WHO should consider developing standard criteria to characterize the genotypes and phenotypes of G6PD variants. This will also help to improve comparability across studies and to inform the classification of new and existing variants, particularly those of uncertain clinical significance. Any new variant should be assigned a tentative percent activity value only if it has been measured at a steady state using a validated quantitative reference test in at least three samples from unrelated males. Other items to be considered include the number of individuals required to examine the distribution of G6PD activity, number of laboratory replica measurements, criteria to define normal reference

values, genetic relationships among cases, methodologies for measuring G6PD activity, phenotypic screening and variant identification, and criteria for including or excluding subjects with concurrent infection or haemolysis. Many variants have now been identified at the molecular level for which important functional properties are unknown. It is desirable to measure at least K_m^{G6P} and thermostability for these and any new variants.

Future research should also address important gaps in knowledge on the risk of severe haemolysis associated with known and potential triggers in already described variants and the identification of other biological factors that might influence haemolytic response (e.g. enzyme activity in reticulocytes).

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Annex 1. List of pre-reads

Nannelli C, Dugué P-A, Bosman A, Luzzatto L. Updating the WHO classification of G6PD variants. Unpublished 2021.

Pfeffer DA, Satyagraha AW, Sadewa A, Price RN, Ley B. Interim analysis of variability in G6PD activity with genetic variant: a systematic review and meta-analysis. Unpublished 2021.

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Annex 2. List of participants

Temporary advisors acting in their personal capacity

Germana BANCONE
Shoklo Malaria Research Unit
Faculty of Tropical Medicine
Mae Sot
Thailand

Daniel A PFEFFER
Menzies School of Health Research
Darwin
Australia

Pierre-Antoine DUGUÉ
School of Clinical Sciences at Monash Health
Monash University
Clayton
Australia

Josef T PRCHAL
Division of Haematology and Genetics
University of Utah
Salt Lake City
The United States of America

Weiying JIANG
Sun Yat-sen University
Guangzhou
Guangdong
China

Mahmoud M SIRDAH
Biology Department,
Al Azhar University-Gaza
Occupied Palestinian territory, including east Jerusalem

Benedikt LEY
Menzies School of Health Research
Darwin
Australia

Olugbemiro SODEINDE
Department of Paediatrics
College of Medicine
University of Ibadan
Ibadan
Nigeria

Lucio LUZZATO
Muhimbili University of Health and Allied Sciences, Dar-es-Salaam, Tanzania and University of Florence
Florence
Italy

Tom VULLIAMY (Chairperson)
Faculty of Medicine and Dentistry
Queen Mary, University of London
London
The United Kingdom of Great Britain and Northern Ireland

Angelo MINUCCI
Laboratory of Clinical Molecular Diagnostics
Institute of Biochemistry and Clinical Biochemistry
Catholic University of Rome
Rome
Italy

Wanchai WANACHIWANAWIN
Division of Haematology, Department of Medicine
Faculty of Medicine Siriraj Hospital
Mahidol University
Bangkok
Thailand

Caterina NANNELLI
University of Florence
Florence
Italy

Rapporteur

Ian C BOULTON
Independent Consultant
London
The United Kingdom

Observer

Mary V Relling
Department of Pharmacy and Pharmaceutical Sciences
Memphis
The USA

WHO Secretariat (Geneva)

Pedro ALONSO
Director
Global Malaria Programme

Jane CUNNINGHAM
Technical Officer
Global Malaria Programme
Diagnostics, Medicines and Resistance Unit

Andrea BOSMAN
Coordinator
Global Malaria Programme
Malaria Director Office

Peter OLUMESE
Medical Officer
Global Malaria Programme
Diagnostics, Medicines and Resistance Unit

Annex 3. Agenda

25 January 2022		
	Session 1	
14:00 – 14:10	Welcome by the Director, Global Malaria Programme Introductions	P Alonso
14:10 – 14:15	Declarations of Interest	A Bosman
14:15 – 14:20	Objectives of the meeting	A Bosman
14:20 – 15:00	Literature search of G6PD activity in males with prevalent G6PD genetic variants Discussion	C Nannelli & P A Dugué
15:00 – 15:25	Interim analysis of variability of G6PD activity with genetic variant: a systematic review and meta-analysis Discussion	D A Pfeffer & B Ley
15:25 – 15:35	*Break*	
15:35 – 16:20	Perspectives on use of current classification of G6PD genetic variants Discussion	G Bancone W Jiang A Minucci J T Prchal M Sirdah O Sodeinde W Wanachiwanawin
16:20 – 17:00	Reflections on updating the classification of G6PD deficiency Discussion	L Luzzatto

27 January 2022		
	Session 2	
14:00 – 14:15	Recap of Day 1 discussions	I C Boulton
14:10 – 15:00	Requirements and process for changing the classification of Classes II and III, including knowledge gaps	*Discussants:* J T Prchal W Wanachiwanawin
15:00 – 15:35	Requirements and process for changing thresholds of Classes I, IV and V, including knowledge gaps	*Discussants:* G Bancone O Sodeinde
15:35 – 16:00	Considerations on 30% detection threshold for G6PD deficiency, including knowledge gaps	*Discussant:* L. Luzzatto
16:00 – 16:10	*Break*	
16:10 – 16:45	Discussion and main points of agreement	T Vulliamy
16:45 – 16:50	Next steps	A Bosman
16:45 – 17:00	Closing remarks	P Alonso