Discussion

Intermediate Glucose-6-Phosphate Dehydrogenase Results

Glucose-6-phosphate dehydrogenase (G6PD) is an enzyme involved in the pentose phosphate pathway. Through the production of NADPH, G6PD helps to protect red blood cells from oxidative damage. Over 200 variants of the *G6PD* gene have been identified and are associated with the clinical spectrum of G6PD deficiency, most importantly the risk of hemolytic anemia under oxidative stress (e.g. with certain drugs, infection, ingestion of fava beans, etc).[1-3]

G6PD deficiency has traditionally been stratified into phenotypic classifications by the World Health Organization (WHO) based on enzyme activity levels: *Class I* (severely deficient variants associated with chronic non-spherocytic hemolytic anemia); *Class II* (severely deficiency variants with <10% enzyme activity associated with acute hemolytic anemia); *Class III* (moderately deficient variants with 10-60% enzyme activity); *Class IV* (normal activity of 60-150%); and *Class V* (increased activity of >150%).[4] While genetic variants are responsible for G6PD deficiency, genotype-phenotype correlations show some degree of overlapping distributions.[5] Furthermore, the definition of 'normal' G6PD activity for the purpose of assigning classifications (and for considering risk of hemolysis with antimalarial drugs) is typically described as the assay and laboratory-specific median G6PD activity derived from unaffected males within its population.[6, 7]

As an X-linked disorder, an affected male carrying a G6PD variant is hemizygous (ie, the one X chromosome contains the variant). Females can be wild type, heterozygous, compound heterozygous, or homozygous, as they have two X chromosomes. Given X chromosome lyonization (ie, inactivation of one of the X chromosomes that occurs in all female cells), females carrying one G6PD variant have a mixed population of red blood cells (independently affected or not affected with G6PD deficiency). For these reasons, females heterozygous with a G6PD variant may also be more likely to have intermediate quantitative G6PD results (between 30% and 80% of normal activity), whereas hemizygous males typically show more distinct deficiency result distributions.

G6PD activity is assessed using either quantitative or qualitative assays. Qualitative G6PD assays are typically designed to identify deficiency states of less than 30% of normal G6PD activity. Qualitative assays – including the fluorescence spot test, colorimetric tests, and lateral flow assays – have less instrument requirements than quantitative assays and are therefore frequently used for screening purposes or in resource limited settings. Qualitative G6PD assays may also be impacted by differences in ambient temperature and patient hematocrit. Therefore, the trade-off with qualitative assays may be less robust performance characteristics compared to quantitative assays.

Some qualitative assays can identify potential intermediate results – for which subsequent quantitative testing may be recommended – while others cannot. In the absence of this ability, some female patients with true intermediate quantitative results may inadvertently receive a normal result when measured by a qualitative assay. In this context, a potential risk for hemolytic anemia susceptibility may go unidentified. This scenario is discussed in several WHO guidelines regarding anti-malarial therapy, for example.[8, 9]

For these clinical reasons, the G6PD CAP Survey occasionally includes specimens with intermediate results to assess their performance on both qualitative and quantitative assays.

In the **G6PDS-A-2020** Survey, specimen **G6PD-01** was designed to obtain a low-range intermediate result. As shown with the quantitative assays, results of 5.25 ± 1.18 U/g hemoglobin (mean ± SD; Point Scientific, 37°C) and 4.12 ± 0.29 U/g hemoglobin (mean ± SD; UDI, 37°C) were observed. These activity levels are distinctly lower than the normal G6PD activity represented in the separate **G6PD-02** specimen. As shown the table below, however, far more participants selected a 'normal' result when using a qualitative assay (48.8%) than did participants who used a quantitative assay (8.3%) for **G6PD-01**.

This is consistent with the expected limitations of qualitative testing as described above.

Table: Summary of G6PD-01 Participant Results and Interpretations

	Quantitative Assay	Qualitative Assay
Normal	8.3% of Participants (6 of 72)	48.8% of Participants (78 of 160)
Intermediate / Sent Out for Further Testing	19.4% of Participants (14 of 72)	31.9% of Participants (51 of 160)
Deficient	72.2% of Participants (52 of 72)	19.4% of Participants (31 of 160)

The G6PD-01 challenge was not graded due to lack of participant consensus (<90% agreement). This event, however, illustrates the difficulty in identifying intermediate results using qualitative tests. Quantitative G6PD reporting practices have been the subject of recent Supplemental Questions associated with the CAP G6PDS Survey and will be described separately in a future Discussion. It is important for laboratorians to keep in mind the diagnostic pitfalls associated with qualitative tests used for assessing G6PD deficiency. While full gene sequencing can provide definitive information on G6PD variant status, this is obviously not practical in most settings.

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