Rev Soc Bras Med Trop 50(2):282-283, March-April, 2017
doi: 10.1590/0037-8682-0003-2017

Letter

Accuracy of CareStart™ G6PD rapid diagnostic test: variation in results from different commercial versions

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Dear Editor,

Glucose-6-phosphate dehydrogenase (G6PD) deficiency (G6PDd) is a genetic disorder resulting in no or low G6PD activity. G6PDd is an X-linked disorder, and more common in men. Patients with G6PDd should not take drugs that cause high oxidative stress such as primaquine, an antimalarial, because of an associated risk of serious side effects, including acute hemolysis and further renal failure. Deaths resulting from these adverse effects, and attributed to primaquine intake, have been reported[1,2]. However, primaquine is the only effective drug for radical cure of *Plasmodium vivax* malaria commercially available.

The gold standard diagnostic techniques used to assess G6PD status are quantitative spectrophotometry (phenotyping) and molecular genotyping. These techniques are expensive and need to be performed in an appropriately equipped laboratory by qualified technicians. These conditions are not usually available in low resource settings. The fluorescent spot test is a rapid screening test recognized by the World Health Organization (WHO), is able to identify severe G6PD deficiency. However, this test displays lower sensitivity in cases of mild and moderate-deficiency phenotypes[3]. G6PD activity needs to be screened in the field to identify G6PDd individuals prior to administering antimalarial treatment, including primaquine. To this end, point-of-care (POC) tests have emerged and are being tested in different malaria endemic regions worldwide. According to experts' consensus, POC tests should have > 95% sensitivity, compared to spectrophotometry, for detection of G6PD enzyme activity that is < 30% that of the normal levels within a specific population[3,4]. To date, the most promising POC platform is CareStart™ G6PD rapid test (Access Bio Inc.). Pilot tests of this G6PD test have been implemented and show that it is easily deployed and more affordable than gold standard G6PDd diagnostic tools. Furthermore, the CareStart™ G6PD rapid test is stored at room temperature and can be performed using capillary blood. Although CareStart™ G6PD rapid test has been demonstrated to have > 95% sensitivity in detecting G6PD deficient phenotypes[5-8], there have been discrepant results. Particularly, low sensitivity among individuals with enzyme activity < 30% has been reported in Cambodia (68%)[9] and more recently in the Brazilian Amazon (61.5%)[10].

To which factor(s) can we attribute this high frequency of false-negative results in G6PD deficient individuals who participated in these studies? A careful reading of the published methods reveals the cause of the discrepancy. Following the manufacturer's instructions, two microliters of blood were added into the sample well, and two drops of buffer into the buffer well. Results were read visually after 10 minutes. According to the reading window, results in all studies were read as follows: G6PD normal for a distinct purple color background, and G6PDd for a background with no color. However, no mention of a pale purple color background appeared in the first batches of CareStart™ G6PD manufacturer's instructions[11]. As a result, a pale purple background was classified as normal only by the Cambodian study, and later by the Brazilian study[9,10]. The most recent CareStart™ G6PD test instructions indicate that no color change or the appearance of a very faint purple color in the reading window within 10 min (borderline results) should be read as deficient[12]. This clarification has resulted in subsequent studies consistently showing high sensitivity[5-8].

Transparency of reporting of studies involving diagnostic accuracy allows us to assess the potential for study bias (internal validity) and to evaluate the generalizability (external validity)[13]. In the case of the CareStart™ G6PD test, with the intended use of determining the need for drug prescription, a diagnostic accuracy study will assess the ability of this POC test to correctly classify patients as having G6PDd. From a patient management perspective, where the individual risk/benefit ratio dictates optimal treatment, knowing the G6PD status of the patient is a prerequisite for safe prescription of 8-aminoquinoline drugs such as primaquine or tafenoquine[3]. Methodology and

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Received 5 January 2017
Accepted 3 April 2017

interpretation of results should be described in enough detail as to allow replication and establish the external validity of a test. This is critical to determine whether decisions about the positivity of the test are highly dependent on the performer's subjectivity. Whether CareStart™ G6PD test results are categorized as either positive or negative depends on manufacturer's instructions, and the sensitivity of the index test is likely to be underestimated if the faint purple color often appears to the reader.

In conclusion, protocols for CareStart™ G6PD test use in the field and systematic reviews need to be standardized for the accurate reading of results. The current recommendation of classifying a faint purple color in the reading window as deficient is the more conservative and safe approach. This avoids the misclassification of moderate G6PDd, most likely to occur in women and in men from areas with mild/moderate phenotypes.

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