ORIGINAL ARTICLE

Noninferiority of glucose-6-phosphate dehydrogenase deficiency diagnosis by a point-of-care rapid test vs the laboratory fluorescent spot test demonstrated by copper inhibition in normal human red blood cells

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Tens of millions of patients diagnosed with vivax malaria cannot safely receive primaquine therapy against repeated attacks caused by activation of dormant liver stages called hypnozoites. Most of these patients lack access to screening for glucose-6-phosphate dehydrogenase (G6PD) deficiency, a highly prevalent disorder causing serious acute hemolytic anemia with primaquine therapy. We optimized CuCl inhibition of G6PD in normal red blood cells (RBCs) to assess G6PD diagnostic technologies suited to point of care in the impoverished rural tropics. The most widely applied technology for G6PD screening—the fluorescent spot test (FST)—is impractical in that setting. We evaluated a new point-of-care G6PD screening kit (CareStart G6PD, CSG) against FST using graded CuCl treatments to simulate variable hemizygous states, and varying proportions of CuCl-treated RBC suspensions to simulate variable heterozygous states of G6PD deficiency. In experiments double-blinded to CuCl treatment, technicians reading FST and CSG test (n = 269) classified results as positive or negative for deficiency. At G6PD activity ≤40% of normal (n = 112), CSG test was not inferior to FST in detecting G6PD deficiency ($P = 0.003$), with 96% vs 90% ($P = 0.19$) sensitivity and 75% and 87% ($P = 0.01$) specificity, respectively. The CSG test costs less, requires no specialized equipment, laboratory skills, or cold chain for successful application, and performs as well as the FST standard of care for G6PD screening. Such a device may vastly expand access to primaquine therapy and aid in mitigating the very substantial burden of morbidity and mortality imposed by the hypnozoite reservoir of vivax malaria. (Translational Research 2014;■:1–12)

Abbreviations: ACD = acid citrate dextrose; CSG = CareStart G6PD deficiency test; FST = fluorescent spot test; G6PD = glucose-6-phosphate dehydrogenase; NADP = nicotinamide adenine diphosphate; RBC = red blood cell

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AT A GLANCE COMMENTARY

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Background

Plasmodium vivax threatens 2.5 billion and causes >100 million clinical attacks, most originating from untreated forms in the liver. These are rarely treated because the only drug, primaquine, causes threatening acute hemolytic in patients having an inborn deficiency in glucose-6-phosphate dehydrogenase (G6PD).

Translational Significance

- We affirm noninferiority of a potentially important new clinical instrument—a G6PD deficiency test suitable for use where most patients with malaria live—compared with the laboratory standard test.
- We detail a novel laboratory technique for such evaluations—copper inhibition of G6PD in normal red blood cell modeling the full range of phenotype heterogeneity among hemizygotes and heterozygotes.

INTRODUCTION

More than 2.5 billion people live at risk of infection by the blood parasite *Plasmodium vivax*, and more than a hundred million suffer clinical attacks every year.[1,2] Although long viewed as a relatively benign infection, reports and studies from endemic areas and in travelers over the past decade reveal an often pernicious and sometime fatal course associated with a diagnosis of vivax malaria.[3,4] This understanding has focused renewed emphasis and interest on long-neglected clinical and public health issues regarding this infection, especially the very difficult problem of glucose-6-phosphate dehydrogenase (G6PD) deficiency and primaquine therapy.[5]

The main clinical and public health problem is the ability of *P. vivax* to place dormant forms in the liver called hypnozoites. These parasites typically cause 3 or more clinical attacks in relatively quick succession in the months after the primary attack, or may do so up to 1 or 2 years later.[6] Some heavily exposed patients suffer up to 20 distinct hypnozoite-borne attacks of vivax malaria within 2 years.[7] Among cohorts in Thailand and Indonesia, the incidence density of first relapse in the 2 months after a primary attack was about 5/person-year.[8-10] Such attack rates approximate those of *Plasmodium falciparum* in the highest risk zones of sub-Saharan Africa.[11] Failure to prevent relapse in vivax malaria results in very high risk of debilitating illness of deepening seriousness and opportunities for onward transmission to others. Nonetheless, most patients diagnosed with vivax malaria do not receive therapy against relapse as a consequence of the rational fear of causing serious harm with primaquine among unscreened patients with G6PD deficiency.[5]

Among the many drugs available to treat the acute attack of vivax malaria, none affect the latent hypnozoites.[12] The only drug registered as safe and effective in preventing relapses is primaquine, and it has been in continuous use since 1952. At therapeutic dosing against relapse, primaquine causes a mild to severe acute hemolytic anemia in patients having an inborn deficiency of G6PD.[13,14] This extraordinarily diverse and complex X-linked trait occurs most frequently where there is endemic malaria transmission, as it may confer some protection against the onset of severe and threatening malaria.[15] About 400 million people are affected, with an average prevalence of G6PD deficiency in malaria endemic nations of about 8%.[16] The blind administration of primaquine to patients diagnosed with vivax malaria is often rationally considered unacceptably hazardous or reckless by providers of malaria treatment services. In impoverished rural settings, patients very often are not provided primaquine therapy as a direct consequence of a lack of access to G6PD screening.

G6PD deficiency as the basis of hemolytic sensitivity to primaquine was described in 1956,[17] and a variety of diagnostic tests for the disorder appeared within a decade. One of the most widely recommended and used has been the fluorescent spot test (FST) described in 1966 by hematologist and pioneering G6PD scientist Ernest Beutler.[18] It has seen several decades of practical and safe use in the developed world, but finds almost no routine application where most patients with malaria live. The reasons include cost, specialized equipment, laboratory skills, temperature sensitivity, and a cold chain for the reagents. Any one of those pitfalls may suffice to prohibit routine use in impoverished tropical settings. The combination of them explains more than 50 years without access to G6PD screening, which in turn accounts for the lack of access to primaquine therapy against vivax malaria for almost all those patients. We consider this deceptively simple problem the likely basis of most clinical attacks of vivax malaria and attendant burdens of morbidity and mortality.

In the present study, we conducted a laboratory-based evaluation of the performance of a new G6PD screening device that appears to have overcome the obstacles to practical use where most patients with malaria live. The CareStart G6PD kit (CSG; AccessBio Inc, New