Proposal for an Evidence Review Group (ERG) on G6PD testing to support increased access to primaquine for radical cure of Plasmodium vivax and for malaria chemoprophylaxis

Briefing paper for the Malaria Policy Advisory Committee (MPAC), 11-13 Sept 2013

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Background

Glucose 6-phosphate dehydrogenase (G6PD) is a housekeeping enzyme expressed by all human cells, which is particularly essential to the integrity and functioning of red blood cells. 186 different mutations in the G6PD gene, found at the q28 locus on the X chromosome, have been identified and can lead to G6PD deficiency mainly through a decrease in the stability of the enzyme. The result is a range of G6PD phenotypes, ranging from severe to mild enzyme deficiency or even normal enzyme activity. Deficiency is mainly manifested in hemizygous males, with heterozygous females displaying varying levels of enzyme deficiency and homozygous deficient females being rare. Over 400 million people worldwide are estimated to be affected by this X-linked disorder, which is geographically closely associated with malaria distribution. Based on mapping surveys, the median prevalence varies from as high as 32.5% in parts of sub-Saharan Africana and the Arabian Peninsula, to below 20% in central and Southeast Asia and to 4-7% in many countries engaged in malaria elimination. However, severe clinical phenotypes are rare and most G6PD-deficient people are asymptomatic, unless exposed to exogenous oxidative stresses like 8-aminoquinoline-based drugs, such as primaquine. The interest in this genetic disorder emerged in the late 1950s and early 1960s, from studies showing G6PD deficiency as the cause of acute hemolytic anemia (AHA) after administration of primaquine (first approved by the US FDA in 1952), and other studies showing the protective effect from severe malaria morbidity associated with this enzyme deficiency.

Progress towards and aspirations of malaria elimination as well new therapeutic options have renewed interest in diagnostic testing for G6PD deficiency and critical examination of the safety of primaquine phosphate for radical cure of vivax malaria (at 0.25 mg base/kg daily for 14 days). In the malaria treatment guidelines, WHO recommends that G6PD testing be done in regions where G6PD deficiency prevalence is relatively high and that P. vivax radical cure treatment regimens are based on the level of G6PD activity. WHO guidelines specifically recommend that for severe G6PD deficiency no treatment with primaquine is given, while patients with mild-moderate G6PD deficiency should receive primaquine treatment at 0.75 mg base/kg body weight weekly for 8 weeks.

Implementing these recommendations is challenging for several reasons:

i) Mapping spatial characteristics of G6PD prevalence is incomplete and can never inform risk at the level of the individual;

1 Howes et al., G6PD Deficiency Prevalence and Estimates of Affected Populations in Malaria Endemic Countries: A Geostatistical Model-Based Map. PLOS Medicine http://www.plosmedicine.org/article/info%3Adoi%2F10.1371%2Fjournal.pmed.1001339

ii) Even if G6PD testing available, distinguishing the degree of severity is not possible with most diagnostic tests;

iii) The clinical risks in exposing individuals with “moderate” G6PD deficient to oxidant substances such as primaquine are not entirely known.

Because of these challenges, and recent examples such as the withdrawal of clorproguanil-dapsone (LapDap™) from the market in 2008 due to its association with post-treatment hemolytic anaemia in G6PD deficient individuals, ³ ⁴ most countries are not deploying primaquine radical cure for P. vivax on a large scale until G6PD point-of-care tests are available or until dosing regimens/therapies are developed, with low toxicity in G6PD deficient individuals.

The alternative drug furthest along the development pipeline, tafenoquine, a long-life 8-aminoquinoine targeting the liver stage of P. vivax, offers the potential for single dose administration, but also carries the threat of hemolysis in G6PD deficient individuals. This has prompted greater mobilization of funds for research on G6PD tests, coordinated by PATH with funding from the Bill and Melinda Gates Foundation, and by Glaxo-Smith Kline, which is developing tafenoquine.

Diagnostic performance of G6PD tests

Available biochemical tests are either quantitative or qualitative and based on either direct or indirect measurement of G6PD activity. Quantitative tests are most often expressed according to WHO classification thresholds of G6PD activity.⁵ Technical requirements range from highly sophisticated laboratory equipment to lateral flow formats. There are five categories of phenotypic tests for identifying patients with G6PD deficiency: i) direct (spectrophotometry and Beutler’s fluorescent spot test; ii) indirect (methaemoglobin reduction test and brilliant cresyl blue or formazan ring tests); iii) cytofluorometric assay; iv) rapid tests Hirono-1-methoxy PMS Sephadex method and WST8/1 methoxy PMS method; and v) rapid point-of-care tests (including Binax Now G6PD and Carestart G6PD). DNA-based genotypic tests are useful for identifying the specific known variants associated with G6PD deficiency, but require either complete sequencing of the gene or extensive information on the G6PD variants present in a particular population. Furthermore, with the exception of three major variants (Mediterranean, A-, Mahidol) the phenotype (clinical characteristics) of the majority of genotypic variants remain unknown, and therefore, the molecular based diagnosis cannot, as yet, tell you about the clinical severity.

The gold standard is the spectrophotometric assay; however, the fluorescent spot test is probably most widely used in the field despite requirements for a water bath incubator, micropipette and UV light. Several more recently commercialized tests, similar to RDTs in format, are on the market or pre-market. The latter tests offer the potential for point-of-care screening, but head to head

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⁵ Class I Severe deficiency (<10% activity) with chronic (non-spherocytic) haemolytic anemia; Class II-severe deficiency (<10% activity) with intermittent haemolysis; Class III mild deficiency (10-60% activity) hemolysis with stressors, only; Class IV non-deficient variant (60-100%), no clinical sequelae; Class V (>100% activity) no clinical sequelae (WHO Working Group 1989; Yoshida et al., 1971).
evaluations in multiple geographical settings are pending and at least one requires use and storage temperatures < 25°C. All tests, including the qualitative dye reduction tests, are reliable in detecting G6PD deficient hemizygous males and homozygous females (who have enzyme activity below 30%); however only cytofluorometric assays detect reliably G6PD deficiency in heterozygous women. Heterozygous women will display varying levels (not necessarily 50 normal: 50 deficient cells) of enzyme deficiency, due to only partial inactivation of one X chromosome, yet may return ‘normal” test results on qualitative tests (that can only identify severe and mild deficiency 0-30-50% of normal activity), but still be at risk of hemolysis under exogenous oxidative stress.

Several tests have been approved by US-FDA for G6PD screening, but the sole point-of-care test was approved as a Class II device to be used as visual screening test for differentiating between normal and deficient G6PD activity levels and as an aid in the identification of people with G6PD deficiency. Therefore, it is not approved to rule in or out G6PD deficiency or to be the sole basis for clinical decisions taken concerning administration of medicines known to induce acute hemolytic anemia in subjects with G6PD deficiency. A recent list of validated quantitative and qualitative tests for G6PD deficiency prepared by Baird et al. (as part of the thematic review on diagnosis and treatment of P. vivax) is given in the Annex 1.

WHO has not reviewed in recent times the diagnostic performance of various types of G6PD testing methods currently on the market nor is it prescriptive regarding specific methods. The International Committee of Standardization in Haematology recommends the fluorescent spot test. Recently, PATH and other groups have compiled inventories of commercially available G6PD tests and are in the process of conducting head-to-head evaluations. WHO would like to critically review these new research findings and determine if results can inform and/or refine current recommendations regarding G6PD testing linked to administration of anti-malarials which can induce hemolysis in G6PD deficient individuals.

Primaquine treatment decisions in relation to G6PD activity

Patients with G6PD deficiency can develop AHA if exposed to 14-days primaquine at 0.25 mg/kg daily dose (for radical cure of P. vivax). In all G6PD deficient patients, a fall in haemoglobin (Hb) is usually seen after 1-3 days of primaquine administration and Hb generally reaches its minimum levels by Day 7. Depending on the pre-treatment Hb level and on the severity of AHA, blood transfusion may be required to avoid worsening of the anaemia, renal failure and death.

6 http://www.accessdata.fda.gov/scripts/cdrh/devicesatfda/index.cfm


Unfortunately, primaquine sensitivity among the many G6PD variants has only been characterized extensively for two variants, Mediterranean and Mahidol, associated with severe and moderate sensitivity. Even variants considered mild, such as African A- can still be associated with severe hemolysis illness after dapsone, and possibly primaquine, administration. Furthermore, there are likely to be other variants, potentially spread heterogeneously in malaria endemic regions. Thus, the real risk of harm of administering primaquine in a given population is largely unknown in most instances. This is the principal reason why testing for G6PD and patient education about symptoms and signs of hemolysis should be done prior to giving primaquine.

Proposal for a WHO Evidence Review Group on G6PD testing to support increased access to primaquine

Several groups are currently evaluating the diagnostic performance of G6PD point-of-care tests compared to the fluorescent spot test and spectrophotometric assays (using capillary and venous blood) and include genotyping specific genetic variants of G6PD deficiency in a range of geographic areas (Thailand, Indonesia, Cambodia). Furthermore, PATH has ongoing evaluations of pre-market tests, and Glaxo-Smith Kline is leading the development of a new bio-sensor, similar to a blood glucose monitor, to measure enzyme activity and detect G6PD deficiency, and support safe tafenoquine treatment. More details on the latter and availability of results from various evaluations will converge around early-to-mid 2014 and therefore WHO/GMP would like to convene an Expert Review Group (ERG) to review the G6PD diagnostic options, their comparative performance and utility in the field, with the aim of guiding G6PD status testing prior to administration of antimalarials, such as primaquine, which induce hemolysis in G6PD deficient patients. On the basis of this review, the ERG would consider whether to recommend G6PD POC tests as part of practical clinical algorithms for use of primaquine for chemoprophylaxis and radical cure of P. vivax.

These draft recommendations, once reviewed by the WHO TEG on Malaria Chemotherapy and the MPAC, could be considered for inclusion in the next updates of the WHO Guidelines for the Treatment of Malaria.
ANNEX 1 - G6PDd validated quantitative assays and commercial providers

<table>
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<tr>
<th>Kit Name</th>
<th>Manufacturer</th>
<th>Veni-puncture</th>
<th>Test Readout</th>
<th>Chemical Basis</th>
<th>Cold Storage</th>
<th>Laboratory Equipment /Skills</th>
<th>Cost/test</th>
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<tr>
<td>Trinity G-6-PDH (kinetic)</td>
<td>Trinity Biotech, Ireland</td>
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<td>Quantitative</td>
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<td>MBK</td>
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<td>BinaxNOW G6PD</td>
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<td>$1.50</td>
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</table>

From JK Baird, N Valecha, S Duparc, N J White, RN Price. Thematic Review on Diagnosis and Treatment of P. vivax (WHO, unpublished) Operational characteristics of a selection of commercially available (and one pre-market) G6PD tests