Expert Review Group Findings: Point-of-Care G6PD Diagnostics

J. Kevin Baird, Ph.D.

Co-Rapporteur
Dr. Ari Satyagraha
Eijkman Institute
Jakarta, Indonesia
This presentation

- Historic context of the primaquine-G6PD problem
- Rationale of this ERG
- Primaquine practice without G6PD screening
- Basic biology of G6PD deficiency
- G6PD diagnostics
- Point of care G6PD diagnostics
- ERG recommendations on G6PD testing with primaquine therapy
The primaquine-G6PD dilemma

• Tens of millions clinical cases of *P. vivax*/year (?)
• Most of those due to relapse (~80% in New Guinea)
• Incidence density of first relapse =5/p-yr in SE Asia
• Most patients relapse 3 to 8 times in SE Asia
• Chronic or repeated acute vivax leads to severe anemia, risk of death
• 400 million (8%) have G6PD deficiency and exposed to vivax malaria
• Daily primaquine carries potentially lethal risk in patients with G6PD deficiency
• Primaquine toxicity drives both fear of it and its inconvenient 14 days of dosing

*Treating against relapse invites risk of harm by the drug, and not treating with primaquine invites risk of harm by the parasite.*
Why an ERG in 2014?!

- Primaquine registered for anti-relapse Rx in 1952
- G6PD deficiency discovered in 1956
- US Army invented primaquine and used it before G6PD deficiency was even known – so who needs screening?
- US Army experience based on mild, self-limiting A-variant, and they had very few poor outcomes (Korean War, 1950-1953)
- WHO (1960s) adopted that view in recommending PQ therapy without G6PD screening
- WHO (until recently) viewed both relapse and treatment against it as not threatening
Guiding primaquine therapy

Primaquine Policy Since 1952
U.S. Army view of G6PD deficiency

Alving et al., Bull WHO 1960
Discovering variable G6PD deficiency
Variable G6PD deficiency

In Vivo Lability of Glucose-6-Phosphate Dehydrogenase in $Gd^A$ and $Gd^M$ Deficiency

SERGIO PIAZZELLI, LAURENCE M. CORASH, DEATRA D. DAVENPORT, JANET MIRAGLIA, and EOBARD L. AMOROS

The Journal of Clinical Investigation Volume 47 1968
But rationalizations continued

- “Reports on large numbers of patients treated with this regimen, even where G6PD deficiency is quite common, indicate this regimen is generally well tolerated and that hemolyisis, when it occurs, is mild and self-limiting.” WHO TGM 1981
  - “No harm done in using primaquine without G6PD screening”

- “It is doubtful if radical treatment of vivax malaria is necessary if the patient lives in an endemic area where transmission of the infection continues and reinfection likely.” WHO TGM 1981
  - “No harm done by withholding primaquine therapy.”
Improved understanding of primaquine threat

• “In patients with the African variant of G6PD deficiency, the standard course of primaquine therapy produces a benign and self-limiting anemia. In the Mediterranean and Asian variants, hemolysis may be much more severe.”
WHO TGM 2010

— “Harm may be done.”
Improved understanding of *P. vivax* threat

- WHO Technical Brief on Control & Elimination of *P. vivax*, 2015
  - “Harm may done by withholding primaquine therapy.”
Plasmodium vivax causes significant morbidity and mortality and poses unique challenges for malaria control and elimination.

Severe cases and deaths due to P. vivax malaria have been reported from all endemic regions.

Testing for G6PD deficiency is currently technically challenging and relatively expensive; hence, many clinicians fear prescribing primaquine to patients of unknown G6PD status. Weighing that risk against the possibility of repeated clinical attacks with attendant risk of debilitating or threatening illness and onward transmission to others is very difficult.
Where feasible all patients should be tested for G6PD deficiency before administering primaquine. Testing for G6PD deficiency in vivax malaria cases should be seen as an integral part of ensuring universal access to diagnosis and treatment.

G6PD testing should be incorporated into treatment guidelines, and services made available, as tools become available (possibly with referral of patients from lower to higher level health facilities).

Point-of-care G6PD testing acknowledged as solving the primaquine-G6PD dilemma.
Evidence Review Group

Point of Care G6PD Testing to Support Safe Use of Primaquine for the Treatment of Vivax Malaria

WHO Geneva, 8-9 October 2014

“The ERG’s over-arching objective was to consider whether to recommend knowing the status of G6PD deficiency of a patient as part of practical clinical algorithm for the use of primaquine for radical cure of vivax malaria, which in most malaria endemic scenarios means adoption of G6PD POC tests.”
## Evidence Review Group

### ERG Members

<table>
<thead>
<tr>
<th>Name</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kevin Baird (Rapporteur)</td>
<td>Indonesia</td>
</tr>
<tr>
<td>Germana Bancone</td>
<td>Thailand</td>
</tr>
<tr>
<td>Gonzalo Domingo</td>
<td>USA</td>
</tr>
<tr>
<td>Tom Douglas</td>
<td>UK</td>
</tr>
<tr>
<td>Marcelo Ferreira</td>
<td>Brazil</td>
</tr>
<tr>
<td>Paul Garner</td>
<td>UK</td>
</tr>
<tr>
<td>Ros Howes</td>
<td>UK</td>
</tr>
<tr>
<td>Sim Kheng</td>
<td>Cambodia</td>
</tr>
<tr>
<td>Marcus Lacerda</td>
<td>Brazil</td>
</tr>
<tr>
<td>Luco Luzzatto (CHAIR)</td>
<td>Italy</td>
</tr>
<tr>
<td>James McCarthy</td>
<td>Australia</td>
</tr>
<tr>
<td>Didier Menard</td>
<td>Cambodia</td>
</tr>
<tr>
<td>Malay Mukherjee</td>
<td>India</td>
</tr>
<tr>
<td>Francois Nosten</td>
<td>Thailand</td>
</tr>
<tr>
<td>Judith Recht</td>
<td>Brazil</td>
</tr>
<tr>
<td>Ari Satyagraha (Rapporteur)</td>
<td>Indonesia</td>
</tr>
<tr>
<td>Bob Taylor</td>
<td>Thailand</td>
</tr>
</tbody>
</table>

### Observers

- Janice Culpepper
- Penny Grewal
- Stephan Duparc
- Justin Green

### WHO Staff

- Andrea Bosman
- Jane Cunningham
- Peter Olumese
- John Reeder
- Pascal Ringwald
- Silvia Schwarte
- Lasse Vestergaard
- Mariam Warsame
Primaquine practice

Primaquine (PQ) anti-relapse therapy and G6PD testing (updated 19.12.2014)
G6PD biology

Red-Ox Equilibria Favoring Hemolysis

Heme-8-QIM

Heinz bodies/Hemolysis

8-QIM

8-QIM+

MetHb

Hb

NADP+

NADPH

G6P

6PG

G6PD

2GSH

GSSG

NADP+

NADPH

G6P

6PG

G6PD
G6PD biology

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Sex</th>
<th>G6PD activity</th>
<th>Phenotypic nomenclature</th>
<th>Primaquine sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>XY – wild type</td>
<td>Male</td>
<td>Normal</td>
<td>Normal</td>
<td>No</td>
</tr>
<tr>
<td>XX – wild type</td>
<td>Female</td>
<td>Normal</td>
<td>Normal</td>
<td>No</td>
</tr>
<tr>
<td>X*Y – hemizygote</td>
<td>Male</td>
<td>&lt;30% of normal</td>
<td>Deficient</td>
<td>Yes</td>
</tr>
<tr>
<td>X<em>X</em> – homozygote</td>
<td>Female</td>
<td>&lt;30% of normal</td>
<td>Deficient</td>
<td>Yes</td>
</tr>
<tr>
<td>X*X – heterozygote</td>
<td>Female</td>
<td>&lt;30% of normal</td>
<td>Deficient</td>
<td>Yes</td>
</tr>
<tr>
<td>X*X – heterozygote</td>
<td>Female</td>
<td>Between 30% and 80% of normal</td>
<td>Intermediate</td>
<td>Possible</td>
</tr>
<tr>
<td>X*X – heterozygote</td>
<td>Female</td>
<td>&gt;80% of normal</td>
<td>Normal</td>
<td>Unlikely</td>
</tr>
</tbody>
</table>

**KEY ISSUE**
NB – The classification of “intermediate” is possible with quantitative testing but does not yet inform the decision of whether to proceed with primaquine therapy or withhold it. IN QUALITATIVE TEST, “INTERMEDIATE” WILL BE CLASSIFIED AS “NORMAL”
G6PD diagnostics

- Qualitative ("semi-quantitative")
  - Glutathione reduction
  - Heinz body formation
  - Tetrazolium dye reduction
  - NADPH fluorescence ("FST"; qualitative gold standard)
- Quantitative
- Cytological
- Genetic
Point of care G6PD diagnostics

- No laboratory skills: Yes
- No laboratory equipment: Yes
- No cold chain: Yes
- Ambient temperature use: No
- Low cost: Yes

![Image of G6PD test strips and package](image)
Heterozygous females & G6PD screening

- Lyonization creates range of G6PD activity from fully deficient to fully normal phenotypes, and all in between
Heterozygous females & G6PD screening

Satyagraha A, et al., PLoS NTD, in press
Heterozygous females & G6PD screening

Baird JK et al., Transl Res, 2014
Heterozygous females hemolyze

15%  1

19%  3

14%  2

26%  4

Mahidol variant
40-60% normal G6PD activity
## Published evidence POC Dx

<table>
<thead>
<tr>
<th>Ref</th>
<th>test</th>
<th>specimen</th>
<th>Gold Std</th>
<th>Threshold</th>
<th># samples</th>
<th># deficients</th>
<th>heterozygo tes</th>
<th>Sensitivity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>WST81 / methoxy PMS</td>
<td>Finger prick/DBS</td>
<td>R and D diagnostics</td>
<td>&lt;60% median of males and females</td>
<td>235</td>
<td>30</td>
<td>all &gt; 10% normal</td>
<td>72</td>
</tr>
<tr>
<td>2</td>
<td>FST</td>
<td>venous</td>
<td>Trinity BioLabs SA/</td>
<td>Median of normal males [10% genotype]</td>
<td>214</td>
<td>23</td>
<td>25</td>
<td>100 (30%) 91 (60%)</td>
</tr>
<tr>
<td>3</td>
<td>FST</td>
<td>venous</td>
<td>Genotype</td>
<td>All normal by FST</td>
<td>461</td>
<td>27</td>
<td>61</td>
<td>All misclassified by FST</td>
</tr>
<tr>
<td>5</td>
<td>Binax NOW</td>
<td>venous</td>
<td>Trinity</td>
<td>4.0 U/gHb</td>
<td>246</td>
<td>50</td>
<td>-</td>
<td>98</td>
</tr>
<tr>
<td>6</td>
<td>Binax NOW</td>
<td>venous</td>
<td>Trinity</td>
<td>&lt;60% median of males and females</td>
<td>356</td>
<td>11</td>
<td>-</td>
<td>54.5</td>
</tr>
<tr>
<td>7</td>
<td>1st gen</td>
<td>venous</td>
<td>Trinity</td>
<td>Lower limit from 174 normal subject (~30% Mean from &gt;4.56 IU/gHb and [Hb] &gt;12 g/Dl)</td>
<td>490</td>
<td>97</td>
<td>-</td>
<td>68</td>
</tr>
<tr>
<td>8</td>
<td>Care Start</td>
<td>venous</td>
<td>Trinity</td>
<td></td>
<td>456</td>
<td>46(&lt;30%)</td>
<td>-</td>
<td>90 (&lt;10%) 84.8 (&lt;30%)</td>
</tr>
</tbody>
</table>
### Unpublished evidence POC Dx

<table>
<thead>
<tr>
<th>Study/PI</th>
<th>Test</th>
<th>Sample Type</th>
<th>Setting</th>
<th>Operator</th>
<th>Reader Assessment</th>
<th>Temp (°C)</th>
<th>Sensitivity/CI (%)</th>
<th>Specificity/CI (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>Prevalence (%)/Sample Size</th>
<th>Reference Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cambodia/Menard</td>
<td>CareStart v2</td>
<td>Venous &amp; Capillary</td>
<td>Mobile lab</td>
<td>technician</td>
<td>2 Independent readers, if discordant, a third reader</td>
<td>26–29</td>
<td>100.0</td>
<td>98.7</td>
<td>92.2</td>
<td>100.0</td>
<td>15.0/392</td>
<td>G6PD Quantitative Trinity Biotech</td>
</tr>
<tr>
<td>Thailand/Banco ne</td>
<td>CareStart v2</td>
<td>Venous</td>
<td>Lab</td>
<td>technician</td>
<td>2 Independent readers, if discordant, a third reader</td>
<td>28–29</td>
<td>87.5</td>
<td>100.0</td>
<td>100.0</td>
<td>89.7</td>
<td>9–18/150</td>
<td>G6PD Quantitative Trinity Biotech</td>
</tr>
<tr>
<td>Thailand/Banco ne</td>
<td>R&amp;D Diagnostic</td>
<td>Venous</td>
<td>Lab</td>
<td>technician</td>
<td>2 Independent readers, if discordant, a third reader</td>
<td>28–29</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>9–18/150</td>
<td>G6PD Quantitative Trinity Biotech</td>
</tr>
<tr>
<td>Indonesia/Saty agraha</td>
<td>CareStart v2</td>
<td>Venous</td>
<td>Field</td>
<td>technician</td>
<td>1 reader, if unsure, another reader</td>
<td>29–34</td>
<td>100.0/ (100.0–100.0)</td>
<td>98.7/ (97.3–100.0)</td>
<td>89.0/ (77.0–100.0)</td>
<td>100.0/ (100.0–100.0)</td>
<td>9.2/260</td>
<td>G6PD Quantitative Trinity Biotech</td>
</tr>
<tr>
<td>Indonesia/Saty agraha</td>
<td>FST Trinity Biotech</td>
<td>Venous in EDTA</td>
<td>Lab</td>
<td>technician</td>
<td>2 readers, if discordant, a third reader</td>
<td>26–29</td>
<td>91.7/ (80.6–100.0)</td>
<td>92.4/ (89.0–95.8)</td>
<td>55.0/ (40.0–70.0)</td>
<td>100.0/ (100.0–100.0)</td>
<td>8.5/260</td>
<td>G6PD Quantitative Trinity Biotech</td>
</tr>
<tr>
<td>Brazil/Lacerda</td>
<td>CareStart</td>
<td>Venous in EDTA</td>
<td>Lab</td>
<td>technician</td>
<td>2 readers, if discordant, a third reader</td>
<td>19–26</td>
<td>61.5</td>
<td>98.3</td>
<td>42.1</td>
<td>99.2</td>
<td>1.9/674</td>
<td>G6PD Quantitative Pointe Scientific</td>
</tr>
</tbody>
</table>

**Sensitivity = classified as deficient/true deficient**

**NPV = true deficient/classified as deficient**

30% of normal activity set cut-off
Unpublished evidence POC Dx

<table>
<thead>
<tr>
<th>Study/PI</th>
<th>Test</th>
<th>Sample Type</th>
<th>Setting</th>
<th>Reader Assessment</th>
<th>Temp (°C)</th>
<th>Sensitivity (%)/CI</th>
<th>Specificity (%)/CI</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>Prevalence/ Sample Size</th>
<th>Reference Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cambodia/Menard</td>
<td>CareStart v2</td>
<td>Venous &amp; Capillary</td>
<td>Mobile lab</td>
<td>2 Independent readers, if discordant, a third reader</td>
<td>26–29</td>
<td>100.0</td>
<td>94.5</td>
<td>36.6</td>
<td>100.0</td>
<td>3.6/419</td>
<td>G6PD Quantitative Trinity Biotech</td>
</tr>
<tr>
<td>Thailand/Banco ne</td>
<td>CareStart v2</td>
<td>Venous</td>
<td>Lab</td>
<td>2 Independent readers, if discordant, a third reader</td>
<td>28–29</td>
<td>90.9</td>
<td>97.4</td>
<td>90.0</td>
<td>97.4</td>
<td>-</td>
<td>G6PD Quantitative Trinity Biotech</td>
</tr>
<tr>
<td>Thailand/Banco ne</td>
<td>R&amp;D Diagnostic</td>
<td>Venous</td>
<td>Lab</td>
<td>2 Independent readers, if discordant, a third reader</td>
<td>28–29</td>
<td>95.5</td>
<td>97.4</td>
<td>91.3</td>
<td>98.7</td>
<td>-</td>
<td>G6PD Quantitative Trinity Biotech</td>
</tr>
<tr>
<td>Indonesia/Saty agraha</td>
<td>CareStart v2</td>
<td>Venous</td>
<td>Field</td>
<td>1 reader, if unsure, another reader</td>
<td>29–34</td>
<td>83.3/ (53.5–100.0)</td>
<td>92.7/ (90.0–95.5)</td>
<td>17.0/ (3.0–30.0)</td>
<td>100.0/ (99.0–100.0)</td>
<td>1.4/350</td>
<td>G6PD Quantitative Trinity Biotech</td>
</tr>
<tr>
<td>Indonesia/Saty agraha</td>
<td>FST Trinity Biotech</td>
<td>Venous in EDTA</td>
<td>Lab</td>
<td>2 readers, if discordant, a third reader</td>
<td>26–29</td>
<td>100.0/ (100.0–100.0)</td>
<td>92.2/ (89.3–95.0)</td>
<td>18.0/ (5.0–31.0)</td>
<td>100.0/ (100.0–100.0)</td>
<td>1.7/350</td>
<td>G6PD Quantitative Trinity Biotech</td>
</tr>
</tbody>
</table>

Sensitivity = classified as deficient/true deficient

NPV = true deficient/classified as deficient

30% of normal activity set cut-off
ERG Recommendation #1

• G6PD status should be ascertained if possible before administering daily primaquine therapy for 14 days to prevent relapses in patients with confirmed acute *P. vivax* or *P. ovale* infection.

*Any G6PD deficient patient may suffer AHA with daily primaquine therapy at any dose*
ERG Recommendation #2

- G6PD qualitative point-of-care tests to identify G6PD non-deficient patients prior to primaquine administration should be >95% sensitive compared to spectrophotometry or equivalent quantitative tests, stable at temperatures expected in tropical settings (35–40°C) and have a negative predictive value of >95% at G6PD enzyme activity levels <30% of normal.

*POC test must reliably detect true G6PD deficient patients and be robust in rural tropics*
ERG Recommendation #3

- Males who have tested or who have a history of testing normal using a reliable G6PD test should receive standard daily primaquine therapy, as they are not expected to experience harmful adverse drug effects.

*POC tests reliably distinguish males who are deficient versus normal*
ERG Recommendation #4

- G6PD qualitative tests will not identify the majority of heterozygous females some of whom may be at risk of developing AHA secondary to primaquine therapy. Therefore, females who test G6PD normal with a qualitative test should only receive daily primaquine therapy if they can be monitored for signs and symptoms of AHA during the first week of treatment.

*POC tests do not reliably exclude female heterozygotes from risk of AHA with daily primaquine therapy*
ERG Recommendation #5

- Male or female patients diagnosed with acute *P. vivax* or *P. ovale* malaria should not receive daily primaquine to prevent relapses when they have tested G6PD deficient. However, these patients may receive a weekly dose of 0.75mg/kg for 8 weeks provided they are under close medical supervision for signs and symptoms of acute hemolytic anaemia during the first 3 weeks of treatment; and provided they have access to health facilities with capacity for safe blood transfusion.

*Eight weekly doses of 45mg primaquine can provoke AHA, but these patients recovered and did not experience further episodes of hemolysis*
Eight weekly doses of 45mg primaquine can provoke hemolysis, but these patients recovered and did not experience further episodes of hemolysis – this evidence viewed as inadequate to fully inform safety of this regimen.
ERG Recommendation #6

• If G6PD status is unknown and testing is not available then a decision to prescribe daily primaquine to prevent relapses must be based on a balanced assessment of the following:
  – i) The available data regarding the local prevalence of G6PD deficiency in the population;
  – ii) The capacity to identify and safely monitor and then manage primaquine-induced hemolytic reactions in the treatment setting;
  – iii) The benefits of treatment in terms of expected reduction in number of relapses

Acknowledges that G6PD screening is not always necessary, and that monitoring may suffice and be warranted when weighed against the threat of relapses
ERG Recommendation #7

• Patients diagnosed with acute *P. vivax* or *P. ovale* malaria and whose G6PD status is unknown may receive a weekly dose of 0.75mg/kg for 8 weeks under close monitoring for signs and symptoms of acute hemolytic anaemia during the first 3 weeks of treatment, with access to health facilities with blood transfusion services.

Acknowledges that G6PD screening is often not available and offers a relatively safer approach to therapy against relapse, while also acknowledging the threat that may be posed by weekly dosing with 45mg primaquine.
What these recommendations deliver

- Acknowledgement of risk of harm with administration of primaquine without knowing G6PD status
- Acknowledgement of risk of harm in withholding primaquine therapy
- Performance standards for point-of-care G6PD devices
- Clear guidance for primaquine therapy with G6PD status being known
- Clear guidance for primaquine therapy with G6PD status being unknown
- Careful balance between striving for safety with primaquine without precluding its use and inviting harm caused by the parasite
- Identifies primaquine-sensitivity in female heterozygotes as a key unknown for research exploration
- Opens the door to commercial competition for a better and less expensive POC for G6PD deficiency