Testing for G6PD deficiency for safe use of primaquine in radical cure of *P. vivax* and *P. ovale*

Webinar presentation to support the dissemination of the policy brief

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At the end of the presentation you will know more about...

- Background, WHO recommendations and the expected benefits
- Primaquine (PQ) and glucose-6-phosphate dehydrogenase (G6PD) deficiency – Particularities in male and female individuals – G6PD testing
- Point-of-care (POC) G6PD testing and safe PQ administration
- Implementation of recommendations at country level
The policy brief contains **guiding principles** and practical advice

- An **algorithm** for qualitative POC G6PD testing and the safe administration of PQ
- Information on the **sourcing** of quality-assured PQ
- A PQ **dosing** table considering available products
- **Checklists** for
  - patient counselling
  - the detection of primaquine-induced acute haemolytic anaemia (AHA)
  - the management of side effects
- Advice on **risk-benefit assessments** to administer PQ without G6PD testing
- Information on quantitative and qualitative **G6PD testing** methodologies (including **preferred product characteristics** for qualitative POC tests)
- Advice on planning and **implementing** G6PD testing at country level
Complete cure of *Pv / Po* infection requires:

- antimalarial medicine to cure the blood-stage infection, *plus*
- primaquine (PQ) to cure the liver-stage infection and prevent relapse

PQ is currently the **only available anti-relapse medicine** – however, its full potential is not used because it produces **dose-dependent AHA in individuals with G6PD deficiency.**

Challenge: G6PD testing often not available at the point of care, and PQ is either:

- given without prior G6PD testing, exposing some patients to the risk of drug-induced AHA, or
- not administered, exposing patients to the risk of repeated relapses with consequent morbidity and contribution to transmission.
WHO recommendations

The G6PD status of patients should be used to guide administration of primaquine for preventing relapse.

- To prevent relapse, treat *P. vivax* or *P. ovale* malaria children and adults (except pregnant women, infants aged < 6 months, women breastfeeding infants aged < 6 months, women breastfeeding older infants unless they are known not to be G6PD deficient and people with G6PD deficiency) with a 14-day course of primaquine at 0.25–0.5 mg/kg body weight daily in all transmission settings.

- In people with G6PD deficiency, consider preventing relapse by giving primaquine base at 0.75 mg/kg body weight once a week for 8 weeks, with close medical supervision for potential primaquine-induced haemolysis.

- When a patient’s G6PD status is unknown and G6PD testing is not available, a decision to prescribe primaquine must be based on an assessment of the risks and benefits of adding primaquine.

- For women who are pregnant or breastfeeding, consider weekly chemoprophylaxis with chloroquine until delivery and breastfeeding are completed; then, on the basis of the woman’s G6PD status, treat with primaquine to prevent future relapse (2).
Expected benefits on two levels

**Individual benefits**
- **Decreased morbidity** due to Pv / Po relapses
- **Reduced risk to develop AHA** in G6PD-deficient patients

**Public health benefits**
- Fewer cases of Pv / Po relapse and AHA will **unburden the health system** through a decreased need for malaria and AHA management (including blood transfusion services)
- Reduced Pv / Po relapse rates will contribute to **reducing transmission** of these parasites (impact will be higher against Pv tropical strains which have higher relapse rates)
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Primaquine, an 8-aminoquinoline derivative, is used since 1950s to treat:

**P. falciparum malaria as gametocytocide**
- Given as a single low-dose medicine at 0.25mg/kg body weight, PQ is well tolerated regardless of the patient’s G6PD status. (Please see [http://www.who.int/malaria/publications/atoz/who_htm_gmp_2015.1.pdf?ua=1](http://www.who.int/malaria/publications/atoz/who_htm_gmp_2015.1.pdf?ua=1).)

**Plasmodium vivax and P. ovale** malaria for radical cure of liver-stage infections
- Given at 0.25-0.50mg/kg body weight daily for 14 days, PQ is safe, well-tolerated and highly efficacious in preventing relapses in G6PD normal patients
- In G6PD deficient patients, however, this 14 days PQ regimen induces a dose-dependent and potentially severe haemolysis in a significant proportion of individuals
350 million people affected globally
Prevalence varies from 3% to 35% in tropical areas

>180 different G6PD deficiency genetic variants

Two of the most prevalent variants represent the two ends of the severity spectrum:

- **Africa A−**: sub-Saharan Africa, and African-Americans (mild)
- **Mediterranean**: Europe, West and Central Asia, and northern India (severe)
AHA severity depends on PQ dose and frequency and the variant of the G6PD enzyme

In less severe G6PD variants

- AHA evident after 1-2 days (oxidant defenses of all older erythrocytes depleted)
- If PQ is continued in African A– variant, haemolysis lessens and haemoglobin concentration rises again, despite further PQ administration: reticulocytes enter the circulation to replace the haemolysed cells
- Young RBCs contain five times more G6PD than oldest RBCs and are hence relatively resistant to the haemolytic effect
- Further haemolysis does, however, occur with higher doses

In the more severe Mediterranean variant

- Haemolysis continues if PQ is not stopped, and life-threatening anaemia may result
The higher the daily PQ doses, the shorter the red cell half-life (left-hand figure)

The higher the daily dose and/or the more severe the variant, the higher the fractional fall (right-hand figure)
Relationship between G6PD genotypes, enzyme activity and sensitivity to primaquine anti-relapse treatment

- G6PD deficiency is an X-linked disorder: males have only one G6PD allele, females two, resulting in two distinct G6PD genotypes in males (wild type and hemizygous), and three in females (wild type, homozygous and heterozygous).

- The five genotypes in males and females translate into three phenotypes:
  - **G6PD normal** (G6PD enzyme activity > 30% of normal, in both male and female individuals)
  - **G6PD deficient** (G6PD enzyme activity < 30% of normal, in both male and female individuals)
  - **G6PD intermediate** (with an enzyme activity varying from 30 to 80% of normal), in heterozygous females only

<table>
<thead>
<tr>
<th>GENOTYPE</th>
<th>SEX</th>
<th>G6PD ENZYME ACTIVITY*</th>
<th>PHENOTYPE</th>
<th>SENSITIVE TO PRIMAQUINE</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>X*Y – hemizygote</td>
<td>Male</td>
<td>&lt; 30% of normal</td>
<td>Deficient</td>
<td>Yes</td>
</tr>
<tr>
<td>XX – wild type</td>
<td>Male</td>
<td>Normal</td>
<td>Normal</td>
<td>No</td>
</tr>
<tr>
<td>X*X – homozygote</td>
<td>Female</td>
<td>&lt; 30% of normal</td>
<td>Deficient</td>
<td>Yes</td>
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<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>

Phenotype determines PQ sensitivity, i.e. likelihood that patient develops AHA after taking PQ.
Qualitative G6PD deficiency testing with currently available POC tests in male and female individuals

Typically, tests have a G6PD enzyme activity detection threshold of 30%:

- Enzyme activity >30%: “G6PD normal”
- Enzyme activity <30%: “G6PD deficient”
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- Implementation of recommendations at country level
Non-G6PD-deficient individuals *: 14-day PQ dose of 0.25-0.5 mg/kg bw daily safe and effective

Heterozygous females: Substantial haemolytic response possible after PQ administration. AHA severity varies from that observed in hemizygous males (if the majority of their red cells are G6PD deficient) to very little haemolysis (if the majority of their red cells are G6PD normal)

*Pregnancy, infants < 6 months: only limited safety data => PQ not recommended
To reduce the risk for haemolysis of individuals who do not have severe variants of G6PD deficiency, an intermittent primaquine regimen of **0.75 mg base/kg body weight weekly for 8 weeks** can be given, under **medical supervision**.

This weekly administration ameliorates the anaemia by allowing haematological recovery after each dose.

**FIGURE 6**
Haemolytic response of the same person after daily and after weekly administration of primaquine

![Graph showing haemolytic response](image)
Algorithm for qualitative POC G6PD testing and the safe administration of PQ to prevent relapse of *Pv* or *Po* malaria

Male and female patients with confirmed *P. vivax* or *P. ovale* malaria (except pregnant women, infants aged < 6 months, women breastfeeding infants aged < 6 months, women breastfeeding older infants unless they are known not to be G6PD deficient)

- Qualitative G6PD testing

- **< 30% G6PD activity**
  - **Female and male: G6PD deficient**
    - Patient counselling
  - **Female: the individual could be G6PD normal or G6PD intermediate**
    - Consider patient as G6PD intermediate with potential risk for haemolysis

- **> 30% G6PD activity**
  - **Male: G6PD normal**
    - 14 days' primaquine regimen (0.25-0.5 mg/kg body weight daily)

- **Female: the individual could be G6PD normal or G6PD intermediate**
  - Patient counselling

8 weeks' primaquine regimen (0.75 mg/kg body weight once a week) under medical supervision

In all cases: mark G6PD status on health records

*More information on risk-benefit assessment, patient counselling and medical supervision is provided in the text.*
Checklists

**Box 1**
**Checklist for Patient Counselling**

Ideally, with the help of adequate product information material (consider local languages, as required), patients should be informed about the following:

- Explain the benefit of primaquine administration.
- Enquire the patient for a medical history of haemolysis.
- Inform the patient about the risk for acute haemolytic anaemia when taking primaquine.
- Instruct the patient to monitor the colour of her or his urine.
- Instruct the patient to stop taking primaquine if her or his urine becomes dark.
- Inform the patient where to seek medical advice if her or his urine becomes dark (the nearest hospital with blood transfusion services).

**Box 2**
**Checklist of Symptoms of Acute Haemolytic Anaemia**

- Back pain
- Dark (red or black) urine
- Jaundice
- Fever
- Dizziness
- Breathlessness

**Box 3**
**Checklist for the Management of Side-Effects**

- Stop administering primaquine. As primaquine is eliminated rapidly, haemolysis is self-limiting once administration is stopped (2).
- Give oral hydration.
- Refer to an inpatient facility.
- Make a clinical assessment.
- Check haemoglobin or haematocrit.
- Check plasma or serum creatinine or urea (blood urea nitrogen) if possible.
- Give a blood transfusion, if necessary, as follows (3):
  - Haemoglobin < 7 g/dL: transfuse
  - Haemoglobin < 9 g/dL with concurrent haemolysis: transfuse
  - Haemoglobin 7–9 g/dL or > 9 g/dL and no evidence of concurrent haemolysis: careful fluid management with monitoring of urine colour.
### Key factors to consider for a **risk-benefit assessment** of primaquine administration **without** G6PD testing

<table>
<thead>
<tr>
<th>CONSIDERATION</th>
<th>MITIGATING FACTORS</th>
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<tbody>
<tr>
<td><strong>Risks</strong></td>
<td></td>
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<tr>
<td>- Acute haemolytic anaemia may occur in patients with unidentified G6PD deficiency</td>
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</table>

*The public health risk–benefit is less favourable in areas with low relapse rates.*

- Epidemiological parameters in areas of intended primaquine use:
  - Low prevalence of G6PD deficiency (including severity of prevalent G6PD variants)
  - Relative low incidence of *P. vivax*

- Health care system:
  - Relatively good access to health care and health facilities
  - Capacity of health services to identify and manage cases of acute haemolytic anaemia, with access to blood transfusion services

<table>
<thead>
<tr>
<th><strong>Benefits</strong></th>
<th></th>
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<tbody>
<tr>
<td>- Relapse prevention or radical cure of <em>P. vivax</em> liver-stage infections</td>
<td></td>
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<tr>
<td>- Reduced vivax transmission and reduced morbidity and mortality due to malaria</td>
<td></td>
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</tbody>
</table>

*The public health risk–benefit is more favourable in areas with high relapse rates.*

- Epidemiological parameters in areas of intended primaquine use:
  - High prevalence of G6PD deficiency (including severity of prevalent G6PD variants)
  - Relatively high morbidity and mortality due to *P. vivax* malaria

- Health care system:
  - Relatively poor access to health care and health facilities
  - Limited capacity of health services to identify and manage cases of acute haemolytic anaemia, with limited access to blood transfusion services
Dosing, administration and sourcing of quality-assured PQ

- PQ is recommended in all transmission settings.
- Tolerability can be improved by taking PQ with food.
- Only primaquine of proven quality and in line with international standards should be used, please see http://www.theglobalfund.org/documents/psm/PSM_ProductsMalaria_List_en/.
- PQ dosing is limited by the **two tablet sizes** currently available at internationally agreed quality standards (7.5 mg and 15.0 mg tablets – the tablets are small, not scored and difficult to cut).
- Patient's adherence is critical (counselling, directly observed treatment (DOT), improved packaging, etc.)

<table>
<thead>
<tr>
<th>PATIENT'S BODY WEIGHT (KG)</th>
<th>DOSE RANGE (MG/KG BODY WEIGHT) PER DAY FOR 14 DAYS</th>
<th>DOSE (MG/KG BODY WEIGHT) PER WEEK FOR 8 WEEKS</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 – &lt; 25</td>
<td>0.25 – 0.5</td>
<td>0.75</td>
</tr>
<tr>
<td>25 – &lt; 50</td>
<td>3.75 – 7.5</td>
<td>7.5 – 15.0</td>
</tr>
<tr>
<td>50 – 100</td>
<td>7.5 – 15.0</td>
<td>30.0</td>
</tr>
<tr>
<td></td>
<td>15.0 – 30.0</td>
<td>45.0</td>
</tr>
</tbody>
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Implementation at country level

G6PD testing should be incorporated into national treatment guidelines, and services made available as tools are developed, possibly with referral of patients from lower to higher level health facilities where both G6PD testing and primaquine can be provided.

Different G6PD testing methodologies can be applied in different settings / levels of the health care system:

- **Qualitative** tests classify an individual as G6PD deficient or G6PD normal according to a specific threshold (typically 30% enzyme activity).
- **Quantitative** tests provide the distinct G6PD enzyme activity of an individual.
1. Laboratory settings

**Quantitative testing:** Spectrophotometric assay and cytochemical assay are mainly in use
- Allow for unambiguous identification of an “intermediate” G6PD status
- Not suitable for routine use in most field settings, as they require a functioning cold chain, laboratory equipment and skilled workers, or are too expensive

**Qualitative testing:** Fluorescent Spot Test (FST)
- Recommended qualitative screening method over the past decade
- Produces visual results within minutes, is affordable, but requires trained staff and necessary equipment, and is hence **not suitable as POC test in the field**

*Note:* Any novel qualitative G6PD tests should be at least equivalent to FST in diagnostic performance
2. Testing at point of care

Qualitative POC lateral flow tests have recently become commercially available:

- require whole blood from a finger-prick
- can be performed and interpreted by health workers at POC
- results in less than 30 minutes

Novel products – still some limitations:

- missing control lines
- subjective interpretation of test results
- incomplete information on thermal stability
- evaluation in studies with small samples sizes
- studies in laboratories / controlled environments
- performed by lab technicians rather than health workers
- only limited evidence on operational aspects and availability of robust, thermostable and easy-to-use POC G6PD tests
In April 2016, the WHO Prequalification team announced expansion to G6PD tests – procedures under development

An Expert Review Panel for diagnostics mechanism has been established by the Global Fund and UNITAID, coordinated by WHO.

**BOX 4**

**PREFERRED PRODUCT CHARACTERISTICS FOR QUALITATIVE POINT-OF-CARE G6PD TESTS**

- The **sensitivity** should be > 95% of that of spectrophotometry or equivalent quantitative tests for detecting G6PD enzyme activity at levels < 30% of normal.

- The **negative predictive value** should be > 95%, i.e. provide a 95% probability that the patient has > 30% normal G6PD activity when the diagnostic test indicates that he or she is not deficient.

- The product should be stable at the **temperatures** expected in tropical settings (30–40 °C).

- The test should have a **visual read-out** that clearly distinguishes between “deficient” and “normal” G6PD activity.
Introducing/expanding a G6PD testing system at country level – Checklist (I)

1. Policy framework

- Include of G6PD testing in national treatment guideline
- Determine level of the health care system where G6PD testing and PQ administration should be effected
- Consider where patients can be assessed for possible PQ-induced AHA
- Consider implementing a referral system from peripheral health facility to a higher level of the health care system (including fluorescent spot test in a laboratory with necessary skills and equipment)
Introducing/expanding a G6PD testing system at country level – Checklist (II)

2. Health care system

- Develop plan for pilot implementation and progressive scale-up, incl. guidance on PQ administration without G6PD testing in areas where POC G6PD testing will initially not be available

- Prepare training materials for health care personnel (test and treat algorithm, job aids for performance and interpretation of G6PD tests, AHA identification, checklist for patient counselling, supervision. Translations!)

- Record G6PD status. Prepare / adapt reporting material for permanent records of the G6PD status of patients

- Manage side effects, particularly PQ-induced AHA (incl. blood transfusion services)

- Behaviour change communication, incl. demand creation among health care providers and patients

- Pharmacovigilance: strengthen monitoring of PQ administration in combination with other AHA-inducing medicines in G6PD-deficient patients.
Introducing/expanding a G6PD testing system at country level – Checklist (III)

3. Quality assurance and quality control of commodities

POC G6PD tests

- Select and procure products according to preferred product characteristics (see Box 4)
- Monitor temperature during shipment, storage and distribution
- WHO is currently setting up a G6PD test prequalification programme. A GF Expert Review Panel for diagnostics has been established.

Primaquine

- Procure anti-relapse medicines in accordance with internationally agreed quality standards
- Organize for pre/post-shipment quality control (WHO prequalified laboratories)
Introducing/expanding a G6PD testing system at country level – Checklist (IV)

4. Resource implications

☐ Consider time requirements for planning, procuring, preparing training, supervision and data management tools, strengthening the referral system and transfusion services

☐ Plan pilot projects to determine the requirements for training and supervising staff

☐ Consider all costs (e.g. products, shipping, storage, distribution, training and supervision, quality control, health systems costs for management of patients with acute haemolytic anaemia)

☐ Identify a funding source for progressive extension of G6PD testing throughout the country
Thank you very much for your attention

Questions or comments? Please contact
Silvia Schwarte (e-mail: schwartes@who.int)