World Health Organization

Friday, 6 March 2015

Time	Session	Purpose	Type
8.30 am 9:30 am	Session 5; Report from Vector Control Advisory Group Nov 2014 meeting *(R Velayudhan/ M Renshaw)* The question of eradication: defining a process for consultation and consensus *(P Alonso)*	For advice For advice	open
11.00 am	**coffee**		
11.30 am	Session 6: G6PD testing to support safe use of anti-relapse therapy for *P. vivax (K Baird)*	For decision	open
1.30 pm	**lunch**		
2.30 pm	Session 7: Updated RTS,S results and JTEG recommendations *(P Smith)* Report from WHO comparison of impact and cost-effectiveness models for malaria vaccines *(V Moorthy)* RTS,S cost-effectiveness and where does it have a role? *(A Ghani)*	For discussion	closed
4.00 pm	**Coffee**		
4.30 pm	Session 7 (cont.): Discussion	For advice	closed
6.00 pm	**End of day**		

Saturday, 7 March 2015

8.00 am – 11.00 am	Finalization of wording of recommendations, and discussion on MPAC plans for 2015	**For decision**	closed

Malaria Policy Advisory Committee Meeting
5–7 March 2015, Geneva, Switzerland
Background document for Session 5

World Health Organization

Update on VCAG activities for consideration by MPAC

LLIN use in areas with substantive resistance. A new paradigm

February 2015, Geneva, Switzerland

Background

WHO has the task of guiding vector-control policies and practices to respond and adapt to the rapidly changing needs of public health. The WHO Vector Control Advisory Group (VCAG) was established in 2013 to serve as an advisory body to WHO on new categories of vector control for malaria and other vector-borne diseases. This group is jointly managed by the WHO Global Malaria Programme (GMP) and the WHO Department for Control of Neglected Tropical Diseases (NTD). VCAG members were selected from a pool of experts, as per the rules of WHO.

The role of VCAG is first and foremost to assess and shepherd the development of new and innovative vector-control paradigms. In this context, a "new paradigm" is understood as a category of intervention or product class whose public health or epidemiological impact is unproven, because the paradigm targets vectors or transmission contexts where the usefulness of vector control is still uncertain (e.g. vector traps for disease management); the paradigm represents new mechanisms for controlling established vectors in defined transmission settings (e.g. transgenic or otherwise modified mosquitoes); or the paradigm represents the gross modification of an existing intervention to the point where it forms a new product class, or where a new epidemiological impact is expected (e.g. products for use in areas of substantive pyrethroid resistance). To evaluate these novel paradigms, VCAG looks at first-in-line prototypes for each intervention class submitted.

In summary, therefore, VCAG has the following functions:

- to review and assess the public health value of new tools, paradigms, approaches and technologies; and
- to make recommendations on their use for vector control within the context of integrated vector management in a disease or multi-disease setting.

WHO/HTM/GMP/MPAC/2015.5

Box 1. Key definitions	
Product	A specific intervention (e.g. long-lasting insecticidal nets).
Prototype	A first-candidate product example of a paradigm that complies to the minimum target product profile for that paradigm.
Paradigm	A group of products that conform to an overarching minimum target product profile in a format that will allow public health (epidemiological) assessment of the prototype to be extrapolated to other products within the group.
Operational setting	The vector space where the product will be used or applied.
Target product profile (TPP)	A detailed technical description that defines the ideal end goals for a product and guides the development process. The TPP summarizes essential and desirable characteristics as well as the specific studies that will supply the evidence for each conclusion about that product.[1]

Products and paradigms distinctions

Several vector-control paradigms are already recommended for use, including indoor residual spraying (IRS), long-lasting insecticidal nets (LLINs) and larvicides. Within each of these paradigms are multiple products, each of which conforms to an overarching minimum target product profile (TPP). For these established paradigms, proof of concept has already been demonstrated, so any subsequent products that meet the minimum TPP do not have to demonstrate public health efficacy. Rather, they are assumed to have equivalency and function in a similar manner to the first-in-class product, unless there is a dramatic change in the underlying vector population. Vector-control products in established categories or paradigms are evaluated through the WHO Pesticide Evaluation Scheme (WHOPES).

Generating evidence for new paradigms

New vector-control products that do not fit the TPPs of established paradigms need to demonstrate their public health value before they can be used for community vector control. VCAG guides the data generation process to maximize efficiency in both time and cost for the paradigm class. If VCAG perceives value in a paradigm, the committee's role is to provide feedback on exactly what studies would need to be undertaken to support this. Once these data have been generated, VCAG reviews the evidence, provides a technical evaluation and refines the TPP (Figure 1). VCAG then presents its assessments of novel paradigms and recommendations to WHO policy-making bodies – the NTD Strategic Technical Advisory Group (STAG) and the GMP Malaria Policy Advisory Committee (MPAC) – on what, if any, public health benefits can be expected from the paradigm and subsequent products within the paradigm that conform to the minimum published TPP.

1. Modified from Vontas et al. (2014) (*1*)

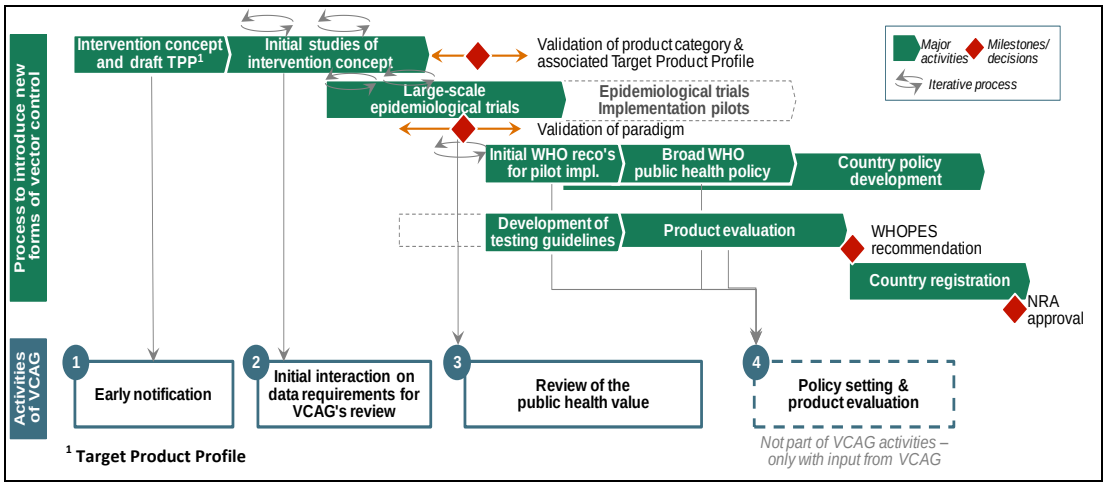

Figure 1 Schematic of the VCAG process

A summary of current and new paradigms for public health vector control

Table 1 (below) lists the current and new paradigms for public health vector control, including their progress in evaluation. IRS and LLINs are divided into two categories: for susceptible and for insecticide-resistant (IR) populations. So far, for IRS, VCAG has reviewed no prototype with a specific claim for efficacy in areas of substantive pyrethroid resistance (although a "non-pyrethroid insecticidal wall lining" may fit in this category).

Table 1: Existing and possible new vector-control paradigms

Parameter	Existing paradigms			New paradigms (supplementary)	
	Larval source management	Insecticide-treated bed nets against **susceptible** vector populations	Insecticide-treated walls against **susceptible** vector populations	Insecticide-treated bed nets against **insecticide-resistant (IR)** vector populations	Insecticide-treated walls against **IR** vector populations
Generic exemplars	Larvicides	LLINs	IRS/wall linings	LLINs controlling IR populations for defined IR mechanism	IRS/wall linings controlling IR populations for defined IR mechanism
Prototype				PermaNet 3 Interceptor G2 (under review)	So far no valid prototype with an explicit claim for IR populations has been reviewed
Operational setting					
Indoors against adults		✓	✓	✓	✓
Outdoors against adults					
Outdoors against immature stages	✓				
Claim: Personal protection	NO	YES	NO	(YES/NO)	NO
Claim: Community protection	YES	YES	YES	YES	YES
WHOPES/ VCAG jurisdiction	WHOPES	WHOPES	WHOPES	WHOPES for long-lasting effect VCAG for IR claim assessment	WHOPES for long-lasting effect VCAG for IR claim assessment
VCAG epidemiological end-point: • personal protection (PP) • community protection (CP)				PP and/or CP[2,3]	CP
Progress of paradigm	Complete	Complete	Complete	VCAG Step 3	To be determined

2. Note: The burden of proof must be structured around the specific claims for each net. Claims should be stated simply, and not overstated. PP function may be retained through the presence of pyrethroid insecticide in some settings, but this will not uniformly be true, and thus will need to be evaluated on a case-by-case basis.
3. The first generation of dual-treated nets are all likely to have pyrethroids as an AI, and some later nets with two non-pyrethroid AIs may not have a PP function. Hence, efficacy claims made for these nets will need to be carefully crafted and individually scrutinized before any broad recommendation can be made for this LLIN against IR vectors paradigm.

Table 1 ctd: Existing and possible new vector-control paradigms

Parameter	NEW paradigms (standalone)						
	Attract and kill baits	Microbial control of human pathogens in adult vectors	Spatial repellents interrupting human–vector contact	Insecticide-treated materials for specific risk groups	Genetic manipulated vectors for reduction of vector population	Vector traps for disease management	Lethal house lures
Generic exemplars	Attractive toxic sugar baits	*Wolbachia*-based bio control	Passive emanator	Insecticide-treated material	Self-limiting gene technology	Traps with lures	Eave tubes
Prototype		*Wolbachia*	Metafluthrin or transfluthrin emanators	Blanket, clothes	OX513A Aedes aegypti (RIDL)	A LOT IN2TRAP	Eave tubes
Operational setting							
Indoors against adults	✓	✓	✓	✓	✓	✓	✓
Outdoors against adults	✓	✓	✓	✓	✓	✓	✓
Outdoors against immature stages					✓	✓	
Claim: Personal protection	NO	NO	YES	YES for specific risk groups	NO	NO	NO
Claim: Community protection	YES	YES	YES	NO	YES	YES	YES
WHOPES/ VCAG	VCAG[a]	VCAG[a]	VCAG[a]	VCAG[a]	VCAG[a]	VCAG[a]	VCAG[a]
VCAG epidemiological end-point: • personal protection (PP) • community protection (CP)	CP	CP	PP and CP	PP	CP	CP	CP
Progress of paradigm (VCAG step)	2	3	3	1	2	3	2

a WHOPES will evaluate any subsequent products under the newly established vector-control paradigms, once data have been generated and reviewed to support the public health use of these products. Risk assessments and development of WHO specifications for products will also be undertaken by WHOPES.

Vector-control products for use in areas of high insecticide resistance

Of the nine paradigms reviewed to date, the paradigm "Vector control products for use in areas of high insecticide resistance" is significantly advanced. For example, new LLINs are being developed by several manufacturers and advocated for use in areas where mosquito vectors are resistant to pyrethroid insecticides. In February 2014, the paradigm "vector control interventions for use in areas of high pyrethroid resistance" was assessed by WHO VCAG.

The paradigm was defined as:

> *a novel intervention or an adaptation of an existing product class that has an overall effect on vectorial capacity and reduces human infection or disease in areas where the local vectors have substantive pyrethroid resistance.*

Under this broad paradigm heading, VCAG has reviewed the data for two insecticide combination/mixture LLINs (Table 1). Permanet 3.0 is a combination LLIN made from a top panel containing deltamethrin and piperonyl butoxide (PBO), a synergist, and side panels containing deltamethrin alone. Interceptor G2 is also a mixture LLIN that contains pyrethroid insecticide plus chlorfenapyr on all parts of the net. Currently, Permanet 3.0 is in Step 3 of the VCAG evaluation, and a prototype of Interceptor G2 will be resubmitted by the manufacturers for VCAG's review, based on initial discussions with the committee in November 2014.

VCAG recommendation to MPAC

VCAG considers combination/mixture LLINs that are designed to have increased effectiveness in areas of high pyrethroid resistance be established as a new paradigm with potential public health value in the face of rising insecticide resistance.

In making this recommendation, VCAG notes that:

1. all nets evaluated under this category must have at a minimum a WHOPES interim recommendation;
2. combination/mixture LLINs will not be equally effective against all types of pyrethroid resistance, particularly those LLINs that contain pyrethroid-based insecticides plus another active ingredient (AI);
3. until combination/mixture nets without a pyrethroid AI become available, a specialist subgroup of VCAG will evaluate and refine manufacturers' claims of product efficacy against highly pyrethroid-resistant vector populations. Substantiated claims will then be supported by VCAG;
4. a guideline for the evidence base needed to substantiate manufacturers' claims has been developed and agreed upon by VCAG. This is attached as Annex 1, for MPAC's reference; and
5. to support the broad paradigm claim above and the insecticide-resistance management aspirations of the *Global plan for insecticide resistance management in malaria vectors* (GPIRM), potential IRS mixtures of AIs (i.e. two or more) for which there is no evidence of pre-existing resistance should also be assessed. As yet, no prototypes for non-pyrethroid IRS mixtures have been submitted for evaluation. Other interventions such as non-pyrethroid combination/mixture wall linings may be reviewed under this paradigm in the near future.

Draft conclusions and recommendations for MPAC's consideration

The VCAG requests the MPAC to consider this background and make a set of recommendations to WHO. The MPAC guidance should include the following main elements:

1. Combination/mixture LLINs designed to have an increased efficacy in areas of high pyrethroid resistance are a new paradigm with potential public health value. New products are needed to address the threat of rising insecticide resistance.
2. Because the efficacy of new LLINs will not be generally applicable to all conditions of insecticide resistance,[4] MPAC at present cannot provide advice on where such nets should be distributed and used.
3. MPAC advises WHO that a detailed, evidence-based plan for the deployment of new LLINs be developed to guide countries and procurement agencies on (*1*) the evidence required prior to net deployment and (2) the operational conditions for where to use such nets.
4. MPAC advises WHO that new combination/mixture LLINs within this paradigm be used only after an appropriate deployment plan is in place.
5. Additionally, MPAC advises WHO that such combination/mixture LLINs should at a minimum have a WHOPES interim recommendation and WHO specifications prior to in-country use.

4. Insecticide-resistance scenarios are defined by (i) frequency as measured by WHO tube tests using discriminative dosages; (ii) mechanisms of resistance (oxidases, esterases and kdr); and (iii) intensity (strength) as measured by LD50 in tube assays or the bottle test.

Annex 1: Guidelines for testing new long lasting insecticide treated nets products to substantiate efficacy claims in areas of high insecticide resistance

Background

New long lasting insecticide treated nets (LLINs) are being developed by several manufacturers and advocated for use in areas where mosquito vectors are resistant to pyrethroid insecticides. In February 2014, the paradigm "vector control interventions for use in areas of high pyrethroid resistance" was assessed by the WHO Vector Control Advisory Group (VCAG). The paradigm was defined as "a novel intervention or an adaptation of an existing product class that has an overall effect on vectorial capacity and reduces human infection or disease in areas where the local vectors have substantive pyrethroid resistance". Under this broad paradigm heading, VCAG has reviewed the data for two insecticide combination/mixture LLINs, and made a recommendation that the combination/mixture nets designed to have increased effectiveness in areas of high pyrethroid resistance be established as a new paradigm with potential public health value.

The current document outlines the evidence that the WHO VCAG would expect to see, to substantiate manufacturers' claims of increased efficacy of combination/mixture LLINs compared with pyrethroid-only LLINs in areas of high insecticide resistance (i.e. resistance ratio [RR] >10-fold).[1] VCAG will convene a specialist subgroup to evaluate and refine manufacturers' claims of efficacy for their products against highly pyrethroid-resistant vector populations. This process is intended to supplement the current WHOPES evaluation procedures for classical LLINs. Further, all combination/mixture LLINs submitted to VCAG with claims of increased effectiveness in areas of high pyrethroid resistance should be well advanced in WHOPES efficacy and safety evaluations and in specification development.

Scope

This document addresses LLINs that are designed to have greater efficacy in areas of high insecticide resistance.[2] Currently, most of these products would address resistance to pyrethroid insecticides and consist of combination/mixture nets, including pyrethroids plus another active ingredient (AI).

Objectives

Next-generation LLINs are likely to be more expensive than current LLINs; hence, control programmes and donors will need information on whether these new nets are more effective at killing (or protecting against) insecticide-resistant populations. Current WHOPES guidelines do not require new LLINs to demonstrate superiority to in-use LLINs. Furthermore, those guidelines recommend that all initial testing of LLIN efficacy be performed on insecticide-susceptible mosquito populations. They also recommend that new nets must demonstrate equivalency to conventional LLINs against susceptible mosquitoes (while recognizing that such populations are increasingly difficult to find and that resistant populations still generate useful data). In reality, new nets, particularly those not containing pyrethroids, may not perform as well as conven-

1. This threshold (RR>10-fold) has been set to exclude mosquito strains with kdr-only based resistance mechanisms.
2. For guidance, at least a 25% improvement should be achieved and the comparator reference strain must be well documented. Manufacturers should specify the percentage improvement with confidence intervals, where the CIs are based on standard errors that reflect the variation between replicates.

tional LLINs against susceptible mosquitoes in WHOPES tests, but may greatly outperform conventional LLINs when resistant mosquitoes are used. New nets are urgently needed to help control pyrethroid-resistant mosquito populations, but it is clear that the current testing guidelines will not generate the data needed to adequately evaluate the performance of these products against these mosquito populations, and further specifications for net evaluation need to be agreed upon.

This document aims to provide guidelines for the minimum data that need to be generated in order to assess whether next-generation LLINs are superior to current LLINs in areas of high resistance. The following assumptions are made:

1. Next-generation LLINs are designed primarily to provide enhanced protection (compared with existing pyrethroid-only LLINs before and after washing) against malaria transmitted by highly pyrethroid-resistant mosquitoes. Hence, all tests should be performed on well-characterized pyrethroid-resistant mosquito populations. The resistance ratio (RR) is pertinent to protection and should be determined.
2. Based on previous studies on fully susceptible pyrethroid vectors, one can assume that if personal protection against highly pyrethroid-resistant mosquitoes is observed, there will be protection against malaria in such settings.
3. Next-generation LLINs are evaluated on their ability to provide enhanced protection or increased mosquito mortality in areas of high pyrethroid resistance rather than on their utility as a resistance-management tool.[3]

Note: Recommendations from this VCAG subgroup on LLIN efficacy against insecticide-resistant populations will relate only to the specific situations tested, and will not be generally applicable to all conditions of insecticide resistance. Nets will need to be appropriately matched to their target area based on the RR and detailed characterization of resistance profiles of local mosquitoes before in-country and regional use.

Evaluating LLIN efficacy against pyrethroid-resistant mosquitoes

Data will be generated using a three-stage approach, to reduce costs and allow the process to be stopped at any stage if increased efficacy is not apparent. These guidelines are intended to provide a general framework for evaluating next-generation LLINs. Detailed standard operating procedures (SOPs) will be attached as annexes to this document.

1. Stage I – Laboratory testing

1.1. Objective

Demonstrate that the next-generation LLIN is significantly better at killing, reducing the reproductive capacity of and/or protecting against pyrethroid-resistant mosquitoes compared to a pyrethroid-only LLIN.

1.2. What is meant by 'significantly better'

i. Next-generation LLINs should be compared to a standard WHOPES-recommended pyrethroid LLIN.[4]

3. Resistance management is a process, and evaluating the utility of individual products in this process will require a burden of evidence that is beyond the scope of this document.

ii. All tests must be performed on at least three characterized industry-standard pyrethroid-resistant mosquito strains, available via the IVCC (Appendix 1),[5] or comply with documentation requirements listed in Section 1.3.

iii. Next-generation LLINs must demonstrate:

- where insect mortality is the expected outcome, at least a 25% increase in mortality compared with pyrethroid-only LLINs, following five replicates for both net types; and
- where insect mortality is NOT the expected outcome, at least a 25% impact on the longevity, blood-feeding and/or reproductive output of the mosquitoes exposed to the new LLIN versus pyrethroid-only LLINs, with statistical significance.

iv. Finally, improvements over current LLINs must be maintained after the requisite number of standardized washes.[4]

Percentage improvement in Phase 1 cone tests has limited operational significance because of poor correlation (or lack of calibration) with field results; however, for guidance, at least a 25% improvement (absolute, not relative) should be achieved using a well-documented reference strain. Manufacturers should specify claims for percentage improvement with confidence intervals (CIs), where the CIs are based on standard errors that reflect the variation between replicate tests.

1.3. What resistance strains to test

i. Standard strains that represent the broad spectrum of major insecticide-resistance mechanisms currently known to exist in mosquito vector populations should be used as the reference test strains for next-generation LLINs. A list of standard strains of insecticide-resistant mosquitoes that may be procured for testing is given in Appendix 1.

ii. At least three strains must be tested, two of which must have major metabolic-resistance mechanisms.

iii. Alternative strains: if alternative strains are used for assessment, the resistance mechanisms must be fully characterized at the time of testing. Results of the resistance profile and evidence demonstrating underlying resistance mechanisms should be documented within the dossier. The resistance level of any strain used for testing must be greater than 10-fold that of a susceptible strain of the same species at the LC50. During all testing, a laboratory susceptible strain must also be run in parallel as a control.

1.4. What method

Robust demonstration of specific beneficial entomological end-points is required; for example, reduction of mosquito life expectancy (increased mosquito mortality), prevention of blood-feeding or reduction of reproductive output. This should be demonstrated by:

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4. Guidelines for laboratory and field testing of long-lasting insecticidal nets. World Health Organisation 2013. ISBN 978 92 4 150527 7.

5. The standard mosquito strains listed in Appendix 1 provide uniform comparators for all studies. Any alternative resistant strains used outside of those listed in Appendix 1 must comply with the documentation requirements described below.

i. Cone bioassay, undertaken as specified in WHOPES guidelines.[6]

- Exposure should be 3 minutes with knockdown recorded at 60 minutes and mortality at 24 hours.
- If an AI has a documented mode of action that does not result in rapid knockdown and kill (e.g. a slow-acting insecticide), the time period for evaluating mortality may be extended; however, a rationale for the testing procedures used must be provided.
- LLINs that do not demonstrate improvements in the cone tests should be tested by tunnel bioassays (below), which will evaluate slow-acting or mechanistically alternative compounds.[7]

ii. Tunnel bioassay, undertaken as specified in WHOPES guidelines.

- Tunnel assays should be used if an AI functions by repellency (requiring testing on free flying insects), or if an AI requires an exposure of greater than 3 minutes to give operationally representative data in cone assays.
- Tunnel tests should use the same strains of resistant mosquitoes as the cone bio-assays.

iii. For products that have a growth regulator AI, measurements of reproductive output (oviposition, fecundity and fertility inhibition) will be needed.

Replicates for cone test

Cone tests should use standardized 2–5 day old non-blood-fed adult females only. The acceptable minimum number of replicates for each mosquito strain is as shown in the table below:

Control 1 (untreated net)	1 net × 5 replicates × 10 mosquitoes = 50 mosquitoes/net
Control 2 (pyrethroid-only LLIN)	4 nets × 5 replicates × 10 mosquitoes = 200 mosquitoes/net
Test nets	4 nets × 5 replicates × 10 mosquitoes = 200 mosquitoes/net
Total	**450 females per strain per new LLIN to be assessed**

- A minimum of one laboratory susceptible strain and three pyrethroid-resistant strains must be tested.
- Sample size calculations should be made in advance of any experimental work, and sample size should be sufficient to demonstrate the minimum effect at a 5% significance level.
- Results should be discarded if mortality on the untreated net exceeds 10%.

6. To test the lifetime of the net longer term trials will be required, but these are not a pre-requisite for simple resistance claims.
7. Tunnel bioassays test may reveal AI toxicity which is not apparent in cone or daytime contact bioassay, as mosquitoes are exposed to the treated nets at night, mimicking natural circadian host-seeking behaviours.

Replicates for tunnel test

Tunnel tests should use standardized 5–8 day old non-blood-fed adult females only. The acceptable minimum number of replicates for each mosquito strain is as shown in the table below:

Control 1 (untreated net)	3 replicates × 50 mosquitoes = 150 mosquitoes/net
Control 2 (pyrethroid-only LLIN)	3 replicates × 50 mosquitoes = 150 mosquitoes/net
Test nets	3 replicates × 50 mosquitoes = 150 mosquitoes/net
Total	**450 females per strain per new LLIN to be assessed**

- Sample size calculations should be made in advance of any experimental work, to clarify the size of effect expected and the minimum effect that can be detected.
- Results should be discarded if mortality on the untreated net exceeds 10%.

Note on mosaic and combination nets: In cases where the sides and top of the net are not treated in an identical manner, data with four nets × five replicates × 10 mosquitoes for each surface type need to be generated. If the proposed mechanism of action is based on the mosquito contacting an insecticide and a synergist located on different parts of the net, accommodation should be made in the guidelines or SOPs for sequential exposure of mosquitoes to the two components; however, this accommodation must not assume that all mosquitoes will contact both parts of the net, and therefore Phase II evaluation is essential to determine efficacy.

1.5. Product quality assurance

Before laboratory, hut or community trials are undertaken, basic quality assurance should be in place to ensure that the products tested meet specifications for quality control (from manufacturers or from WHO, if available).

When supplying the product for testing, manufacturers should provide a certificate that states that the product meets their or WHO specifications for quality control. Quality assurance of the nets by high performance liquid chromatography (HPLC) or gas chromatography (GC) should also be undertaken before the products are tested. Independent physical and chemical analyses of the products for compliance with specifications in an accredited, qualified laboratory may be required before efficacy testing.

All net testing should be undertaken on LLINs that have been washed once and left for the WHOPES recommended regeneration time (or the time specified by the companies against insecticide-susceptible strains), in order to correct for variations in insecticide availability due to storage conditions for the nets.

2. Stage 2 – Experimental hut studies

If the new LLIN product demonstrates significant increased efficacy compared to the standard LLIN against all or most of the resistant strains tested in the laboratory, Stage 2 experimental hut studies should be initiated.

2.1. Objective

Demonstrate that the (holed) new LLINs (as specified in current WHOPES guidelines[1]) are significantly better at inducing mortality or preventing blood-feeding than a standard LLIN (or at

reducing fecundity and fertility of the mosquitoes if a growth regulator is involved) against local highly resistant mosquitoes.

2.2. Site criteria

Experimental hut studies need to be conducted in areas where the mosquito population has high levels (RR >10-fold) of well-characterized pyrethroid resistance. For data to be accepted, the resistance profile and species composition of the site must be determined immediately before, or at the same time as, the trial.

This profiling must include the following:

a. WHO diagnostic dose assays for pyrethroids (deltamethrin and permethrin as minimum).

b. LC50 for all AIs incorporated into the net. A fully susceptible strain should be used as the standard for calculating of the RR of the field population. (If *Anopheles gambiae* s.s. is the local vector, the Kisumu strain should be used.)

c. If a synergist is being tested, effect of pre-exposure to the synergist on insecticide mortality needs to be recorded. For PBO this should be a 1-hour exposure to 4% PBO in a standard WHO bioassay.

d. A baseline of the species composition (including sibling species defined by molecular markers) of vectors entering the experimental huts before the study.

e. A minimum of 500 mosquitoes should be tested for points a, c and d above.

f. Cone bioassays testing 1 × washed and regenerated pyrethroid-only LLINs with local mosquito vectors must be performed before the study.

g. At least 100 adult females (2–5 day old non-blood-fed, non-exposed to insecticides) should be preserved in RNA*later* at the start of the study for future follow-up of resistance mechanisms, if required.

Note on study sites: Suitable study sites will have a vector population that has an RR >10-fold for one or more pyrethroids at the LC50 level when compared to the standard Kisumu strain. Cone tests must also show >50% of mosquito survival against the standard LLIN. Tests undertaken in areas with lower level resistance cannot be used to substantiate product claims against operationally significant pyrethroid-resistant populations.

2.3. Methods

Methodology follows WHOPES guidelines for testing LLINs at the experimental hut level,[8] and the same parameters are calculated (deterrency, induced exiting, blood-feeding inhibition, personal protection and mortality). If sterilizing properties are to be recorded, blood-fed mosquitoes from huts using both net types need to be kept alive and the fertility or fecundity recorded. Additional outcomes may be considered and introduced, depending on the claim of the manufacturer.

Species composition of alive and dead mosquitoes should be determined if there are multiple sympatric vectors, in order to evaluate whether the net is equally effective against all.

8. Guidelines for laboratory and field testing of long-lasting insecticidal nets. World Health Organization 2013. ISBN 978 92 4 150527 7.; pp 14 - 28

Trials should be undertaken in at least three geographically separated locations with distinct vector populations, to assess whether the product is effective at multiple sites.

The trial must include comparison with a WHOPES-recommended LLIN.

3. Stage 3 – Large-scale field trials

The format of the large-scale field trials (i.e. community trials) will depend on whether the mixture/combination LLIN functions through personal protection of the end user or relies predominantly on creating a community effect.

i. Fast acting and repellent compounds will maintain personal protection of the end user; thus, evaluation at a household level, using a household randomized design, will be sufficient.

ii. For all other modes of action, including slow action, epidemiological evidence will be needed due to a loss of personal protection. A community-scale cluster randomized trial design will be required for slow-acting or non-repellent insecticides,[9] or products that are expected to affect mosquito fecundity or fertility.

3.1. Study design for LLINs that work through personal protection

3.1.1. Objectives

It is necessary to demonstrate that, under field conditions, the new product significantly reduces the number of blood-fed mosquitoes collected resting and exiting houses, compared to a pyrethroid-only LLIN.

3.1.2. Study methods

New products that offer personal protection can be tested at the household level with a household randomized control design. This type of trial is suitable, for example, for nets with a rapid-acting insecticide plus a synergist.

Pre-trial considerations

Potential sites need to be characterized prior to trial to ascertain the following:

a. WHO diagnostic dose assays for pyrethroids (deltamethrin and permethrin as minimum).
b. LC50 for all AIs incorporated into the net. The Kisumu-susceptible strain should be used as the standard for calculation of the RR of the field population if the local vectors are *An. gambiae* s.s.
c. If a synergist is being tested, effect of pre-exposure to the synergist on insecticide mortality needs to be recorded. For PBO, this should be a 1-hour exposure to 4% PBO in a standard WHO bioassay.
d. A 3-month baseline of the species composition (including form for *An. gambiae* s.s) of malaria vectors at the field trial site before the study, which should be a minimum of 3 months.

9. Pyrethroid lose their repellency action against pyrethroid resistant populations and therefore combining a pyrethroid with a non-repellent insecticide or synergist would not allow a trial at household level to be sufficient test.

e. A minimum of 100 mosquitoes should be tested for points a to c above.

f. Cone bioassays on 1 × washed and regenerated pyrethroid-only LLINs and local vectors must be performed before the study.

g. At least 100 adult females (2–5 day old non-blood-fed, non-exposed to insecticides) should be preserved in RNA*later* at the start of the study for future follow-up of resistance mechanisms, if required.

Trial procedures

After collection of the baseline data described above, the new and standard net types should be randomly assigned to households, and quarterly indoor and exit collections made over a transmission season. Mosquito densities will be compared between a reference pyrethroid LLIN (positive control) and the candidate LLIN. Additionally, the mosquito densities should be noted before and after the intervention in indoor and exit collections, as well as the physiological status of female mosquitoes and any instances of delayed mortality.

Data will only be considered for trials that have been conducted in an area with documented >10-fold pyrethroid resistance, and where the resistance status has been determined at the time of the trial.

3.2. Study design for LLINs that work only through community protection

For LLINs that work at the community rather than the individual level and that do not offer personal protection, full-scale epidemiological trials will be needed, so a cluster randomized design will be applicable.

Indicators of epidemiological outcome could include incidence of malaria through active case detection, passive case detection, serology or point prevalence of infection. Entomological outcomes (e.g. human landing catch, entomological inoculation rate (EIR) and parous rate) should also be considered.

The design and analysis of these trials should be based on methods appropriate for cluster randomized trials, and standard errors and significance tests should be estimated accordingly.

To facilitate assessment and to standardize testing between products and between independent trials of the same product, all efficacy testing should be completed according to the SOPs and example trial formats available with this document through WHO VCAG.

Appendix 1

Standard insecticide-susceptible and resistant strains used by industry for insecticide development and available as standards for testing via LITE/IVCC.

Name	Species	Country of origin	Phenotype	LC50 Deltamethrin (µg/ml)	Kdr	Ace
Kisumu	*Anopheles gambiae*	Kenya	Susceptible	0.020	0	0
Kisumu Rdl	*Anopheles gambiae*	Kenya	Dieldrin resistant	To be determined	0	0
Akron	*Anopheles gambiae*	Benin	Carbamate resistant	To be determined	0.1	0.5
VK7	*Anopheles gambiae*	Burkina Faso	DDT resistant	0.260	0.4	0
Tiassale	*Anopheles gambiae*	Cote d'Ivoire	Pyrethroid resistant	1.590	0.9	0.4
Moz	*Anopheles arabiensis*	Mozambique	Susceptible	To be determined	0	0
New Orleans	*Aedes aegypti*	United States of America	Susceptible	0.004	0	n/a
Cayman	*Aedes aegypti*	Grand Cayman	Pyrethroid, carbamate and DDT resistant	9.290	0.7	n/a
FuMoz	*Anopheles funestus*	Mozambique	Pyrethroid and carbamate resistant			

List of SOPs to collect for efficacy testing, based on guidelines above.

1. Sample size calculations for claims of percentage improvement
2. Cone bioassay
3. Tunnel bioassay
4. Measurements of reproductive output (fecundity and fertility)
5. Conducting baseline species composition
6. WHO diagnostic dose assays for pyrethroids
7. Calculating RR
8. LC50s for AIs
9. Cone bioassays on local vectors
10. RNA*later* sample preservation
11. WHOPES guidelines for testing LLINs at the experimental hut level, including measurements of reproductive output (fecundity and fertility) and species composition of alive and dead mosquitoes
12. SOPs for community-level trials for personal protection and for community protection/epidemiological outcome

Reference

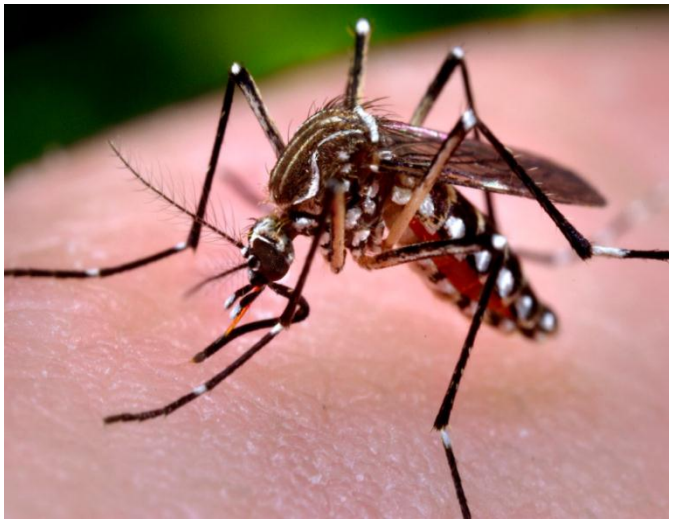
1 Vontas J, Moore S, Kleinschmidt I, Ranson H, Lindsay S, Lengeler C et al. Framework for rapid assessment and adoption of new vector control tools. Trends Parasitol. 2014;30(4):191-204 (http://www.ncbi.nlm.nih.gov/pubmed/24657042, accessed 19 February 2015).

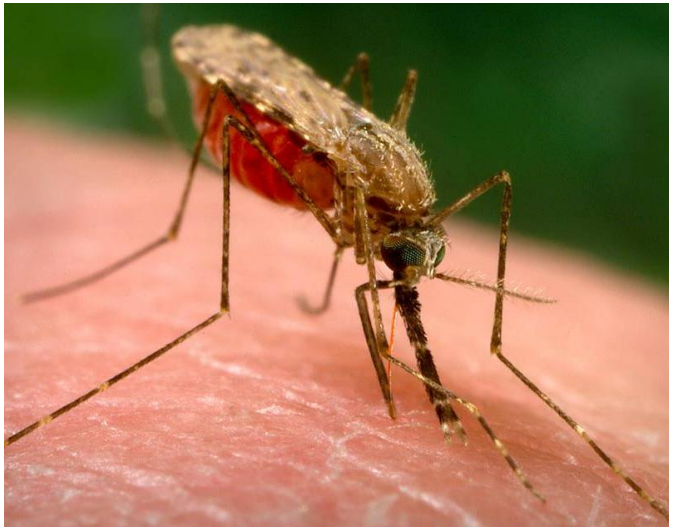
World Health Organization

The WHO Vector Control Advisory Group (VCAG)

…providing a pathway forward for novel paradigms in vector control.

A JOINT ACTIVITY OF NTD AND GMP





VCAG provides a pathway for new paradigms

- **VCAG was established in 2013 to serve as an advisory body to WHO on new categories of vector control for malaria and other vector-borne diseases.**
- **Members were selected through screening from a pool of expert applicants.**

Functions:

1. **To review and assess the public health value, "proof of principle" (epidemiological impact) of new tools, approaches and technologies; and**

2. **To make recommendations on their use for vector control within the context of integrated vector management in multi-disease settings.**

(www.who.int/neglected_diseases/vector_ecology/Operational_procedures_for_VCAG.pdf)

Activities of VCAG

ACTIVITIES OF VCAG

1. Early notification
2. Initial interaction on data requirements for VCAG review
3. Assessment and review of the public health value

Concept development → Proof of concept: Entomological outcomes → Proof of Principle: Large scale epidemiological trials

NEXT STEPS *for 'next-in-line' products*

- Development of testing guidelines (VCAG)
- **WHOPES Testing and Evaluation**

OUTCOME

Validated paradigm & Target Product Profile

NEXT STEPS

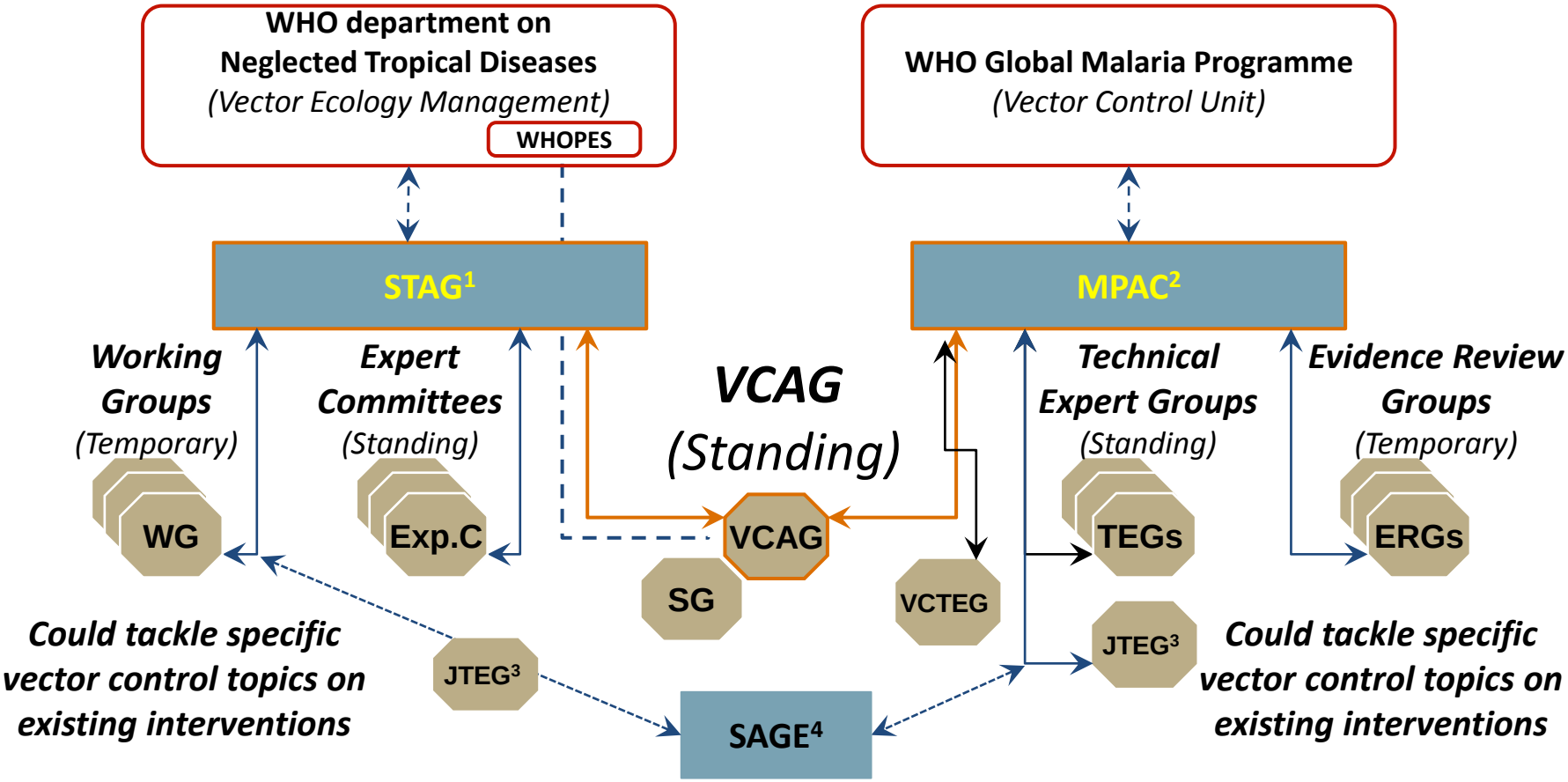
4. **WHO Policy Setting (GMP/NTD)**
   Recommendations on piloting intervention and for health policy

Advisory Groups
MPAC (malaria)
STAG (NTDs)

COUNTRY POLICY, PRODUCT REGISTRATION, PROCUREMENT AND USE

Structure of VCAG

VCAG is a standing group providing technical advice to STAG[1] and MPAC[2] on new forms of vector control



1. Strategic and Technical Advisory Group for neglected tropical diseases 2. Malaria Policy Advisory Committee
3. Joint Technical Expert Group 4. Strategic Advisory Group of Experts on Immunization

WHOPES vs VCAG

	VCAG	WHOPES
	Innovative vector control paradigms	Innovative products from established vector control paradigms (eg. IRS, LLIN, mosquito larvicides, space spraying products)
Scope	Assesses paradigms claims through “first in line” prototype.	Evaluates individual product claims for commercially produced pesticides
Evaluation	Efficacy: Entomological & Epidemiological Safety: Requires risk assessment Other Parameters including TPP, user compliance/acceptability, economic feasibility, manufacturing sustainability and strategic/policy role	Efficacy: Entomological data Safety: Requires risk assessment Quality: WHO Specifications developed through JMPS
Data	Published and unpublished data submitted by innovator	Data from WHOPES supervised efficacy trials, and manufacturer (safety, quality and some efficacy)
Outcome	Recommendations on public health value of the paradigm and prototype to policy setting groups (MPAC/NTD-STAG)	Recommendations on efficacy, safety/risk and quality standards PHPs , used by member states for product registration and procurement

WHOPES evaluates products from existing paradigms

Parameter	Existing Paradigms		
	Larval source management	Insecticide treated bed nets (susceptible vectors)	Insecticide treated walls (susceptible vectors)
Generic Exemplars	larvicides	LLINs	IRS/wall linings
Prototype			
Operational setting			
Indoors against adults		✓	✓
Outdoors against adults			
Outdoors against Immatures	✓		
CLAIM: Personal protection	NO	YES	NO
CLAIM: Community protection	YES	YES	YES
WHOPES/ VCAG	WHOPES	WHOPES	WHOPES
Progress of paradigm	complete	complete	complete

VCAG has reviewed 16 submissions;

VCAG has established 8 new paradigms for vector control

In Feb 2014, VCAG assessed the paradigm

Vector-control products for use in areas of high insecticide resistance

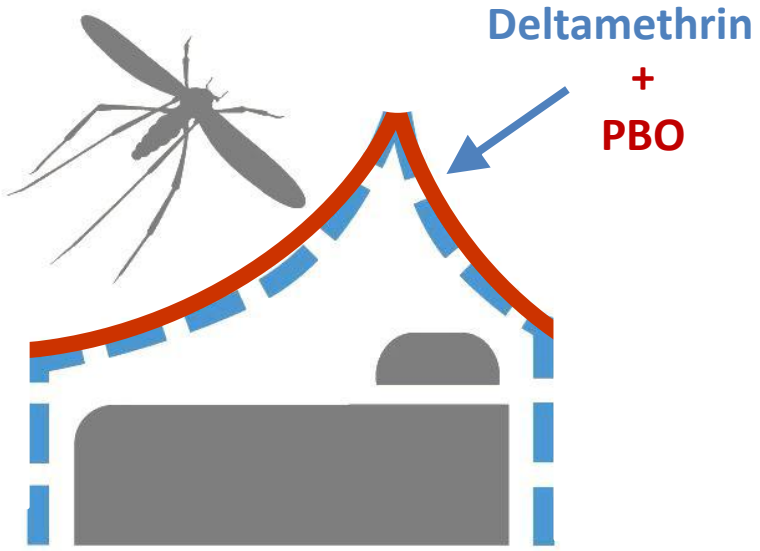
Prototype: Permanet® 3.0

Description of paradigm:

- a novel intervention or an adaptation of an existing paradigm.
- reduces vectorial capacity
- reduces infection/disease in humans in areas where the local vectors have substantive pyrethroid resistance

Prototype: Permanet 3.0

- combination LLIN
- sides: deltamethrin only
- top: deltamethrin + piperonyl butoxide (PBO)



PBO is a synergist that enhances effects of pyrethroids by inhibiting metabolic detoxification enzymes

Prototype: Permanet® 3.0

Prototype claims: in areas of high pyretheroid resistance,

PBO + pyrethroid = ↑ mosquito death & ↓ human infection

Supporting evidence:

Percentage improvements with PN3 compared to pyrethroid only LNs

Reference	Page # in dossier	Country	Site	Outcome measure	% improvement observed with PermaNet® 3.0
Adeogun et al (2012b)	16-24	Nigeria	Ikorudu	% increase in mortality, relative to Olyset®	26
			Kainji		24
Koudou et al (2013)	26-43	Ivory Coast	Bouaké	% increase in mortality cone test on roof with wild mosquitoes after 12 months use, relative to PermaNet® 2.0	63
			Tiassalé		36
Stiles-Ocran (2013)	44-71	Ghana	Chirano area	% reduction in density (measured by indoor resting catches) from baseline to post-intervention, relative to PermaNet® 2.0	39-84
				% reduction in density (measured by human landing catches) from baseline to post-intervention, relative to PermaNet® 2.0	25-63
Awolola et al (2013)	72-92	Nigeria	Ogun State	% reduction in mean household density, relative to PermaNet® 2.0	27
				% increase in mean mortality, relative to PermaNet® 2.0	56
				% increase in EIR reduction, relative to PermaNet® 2.0	28
Adeogun et al (2012a)	94-107	Nigeria	New Bussa	% increase in mortality from 0 washes to 20 washes, relative to Olyset®	36-43
				% increase in blood feeding inhibition from 0 washes to 20 washes, relative to Olyset®	17-24

Recommendations for the prototype:

- VCAG supported the manufacturer's claim of increased bioefficacy compared with pyrethroid only LLINs in areas where mosquitoes have metabolic resistance mechanisms.
- WHOPES phase 3 evaluation towards full recommendation should be completed.
- Any implementation should include resistance monitoring of mechanisms of resistance.

Recommendation made to MPAC

VCAG considers combination/mixture LLINs that are designed to have increased effectiveness in areas of high pyrethroid resistance to be a new paradigm with potential public health value in the face of rising insecticide resistance.

Parameter	New paradigms	
	Insecticide treated bed nets (resistant vectors)	*Insecticide treated walls (resistant vectors)*
Generic Exemplars	**LLINs for specific IR populations**	*IRS/linings for specific IR populations*
Prototype	**PermaNet 3 Interceptor 2**	*To be determined*
Operational setting		
Indoors: adults	✓	✓
Outdoors: adults		
Outdoors : Immatures		
CLAIM: Personal protection (PP)	**YES/NO**	*NO*
CLAIM: Community protection (CP)	**YES**	*YES*
WHOPES/ VCAG	**WHOPES for long-lasting effect; VCAG for IR claim**	*WHOPES for long lasting effect; VCAG for IR claim*
VCAG epidemiological endpoint: Personal (PP) or Community (CP)	**PP and/or CP**	*CP*
Progress of paradigm	**VCAG Step 3**	*TBD*

Additional considerations from VCAG

1. All nets evaluated under this category must have at a minimum a WHOPES interim recommendation;
2. Combination/mixture LLINs will not be equally effective against all types of pyrethroid resistance, particularly those LLINs that contain pyrethroid-based insecticides plus another active ingredient (AI);
3. Until combination/mixture nets without a pyrethroid AI become available, a specialist subgroup of VCAG will evaluate and refine manufacturers' claims of product efficacy against highly pyrethroid-resistant vector populations. Substantiated claims will then be supported by VCAG;
4. A guideline for the evidence base needed to substantiate manufacturers' claims has been developed and agreed upon by VCAG.
5. To support the broad paradigm claim above and the insecticide-resistance management aspirations of the *Global plan for insecticide resistance management in malaria vectors* (GPIRM), potential IRS mixtures of AIs (i.e. two or more) for which there is no evidence of pre-existing resistance should also be assessed. As yet, no prototypes for non-pyrethroid IRS mixtures have been submitted for evaluation. Other interventions such as non-pyrethroid combination/mixture wall linings may be reviewed under this paradigm in the near future.

Draft conclusions for MPAC's consideration

1. Combination/mixture LLINs designed to have an increased efficacy in areas of high pyrethroid resistance are a new paradigm with potential public health value. New products are needed to address the threat of rising insecticide resistance.
2. Because the efficacy of new LLINs will not be generally applicable to all conditions of insecticide resistance, MPAC at present cannot provide advice on where such nets should be distributed and used.
3. MPAC advises WHO that a detailed, evidence-based plan for the deployment of new LLINs be developed to guide countries and procurement agencies on (*1*) the evidence required prior to net deployment and (2) the operational conditions for where to use such nets.
4. MPAC advises WHO that new combination/mixture LLINs within this paradigm be used only after an appropriate deployment plan is in place.
5. Additionally, MPAC advises WHO that such combination/mixture LLINs should at a minimum have a WHOPES interim recommendation and WHO specifications prior to in-country use.

Insecticide-resistance scenarios are defined by (i) frequency as measured by WHO tube tests using discriminative dosages; (ii) mechanisms of resistance (oxidases, esterases and kdr); and (iii) intensity (strength) as measured by LD50 in tube assays or the bottle test.

Summary

- VCAG will continue to assess new paradigms for vector control including addressing the challenges of insecticide resistance management (across all vectors).
- Certain new paradigms are specifically needed to address specific population groups or vectors in targeted niches.
- Provides a predictable and defined process by which new forms of vector control can be introduced into public health practice
- Providing a forum for dialogue and guidance to innovators on evidence requirements early in the process to reduce risks. Reducing uncertainty for innovators through this clarification.
- VCAG also evaluates non-insecticidal vector control tools and will give advice to WHO on their use.
- Accelerating the process of public health implementation of new forms of vector control

Paradigm or Product	Stage	Status	Operational use
Resistance targeting product • PermaNet 3.0	VCAG Step 3 (paradigm cleared)	Recommendation to MPAC.WHOPES interim recommendation (approved) Full trial in progress	LLIN for control of pyrethroid resistant malaria vectors
Microbial Control • Wolbachia	Step 2-3	Large scale trials in progress	Control of dengue transmission in endemic regions
Spatial Repellent	Step2-3	Large scale trials in progress	Repels vectors indoors, interrupting malaria transmission
Genetic manipulation • Oxitec (Aedes)	Step 2-3	Large scale trials in progress In negotiation with Global Health Investment Fund	Population reduction through genetic means to stop dengue transmission
Vector traps for disease management • In2Trap (dengue control)	Step 2	Pilot testing in programmes In negotiation with Global Health Investment Fund	Controls adult and immature vectors of dengue, targets cryptic breeding sites
Lethal House Lures • Eave tubes and bricks	Step 2	Pilot testing in progress In negotiation with Global Health Investment Fund	Kills malaria vectors entering houses through eaves
Attract and kill baits • Attractive Toxic Sugar Bait	Step 2-3	Large scale trials approved (IVCC)	Attracts and kills malaria vectors indoors and outdoors
Treated materials for specific groups	Step 1	In development	Outdoors in disaster situations, malaria

Activities on going:

The fourth VCAG meeting will be held in November 2015.

In anticipation of possible evolution of the paradigms/prototypes discussed VCAG is also currently developing the following:

Risk assessment of Wolbachia
Establishment of Subgroup of experts on resistance management claims
Guidance for evaluating traps

Parameter	NEW Paradigms (stand-alone)						
	Attract and kill baits	Microbial control of human pathogens in adult vectors	Spatial repellents interrupting human-vector contact	Insecticide treated materials for specific risk groups	Reducing vector populations through genetic manipulation	Vector traps for disease management	Lethal house lures
Generic Exemplars	Attractive Toxic Sugar Bait	Wolbachia-based bio control	Passive emanator	Insecticide treated material	Self-limiting gene technology	Traps with lures	Eave tubes
Prototype	Bait station	Wolbachia	Metofluthrin/ transfluthrin emanators	Blankets/ Clothes	OX513A Aedes aegypti	A LOT IN2TRAP	Eave tubes
Operational setting							
Indoors: adults	✓	✓	✓	✓	✓	✓	✓
Outdoors: adults	✓	✓	✓	✓	✓	✓	✓
Outdoors : Immatures					✓	✓	
CLAIM: Personal protection (PP)	NO	NO	YES	YES	NO	NO	NO
CLAIM: Community protection (CP)	YES	YES	YES	NO	YES	YES	YES
WHOPES/ VCAG	VCAG	VCAG	VCAG	VCAG	VCAG	VCAG	VCAG
VCAG epidemiological endpoint:	CP	CP	PP & CP	PP	CP	CP	CP
Progress of paradigm (VCAG Step)	2	2/3	2/3	1	2/3	2/3	2

Thank you

Malaria Policy Advisory Committee Meeting
5–7 March 2015, Geneva, Switzerland
Background document for Session 6

World Health Organization

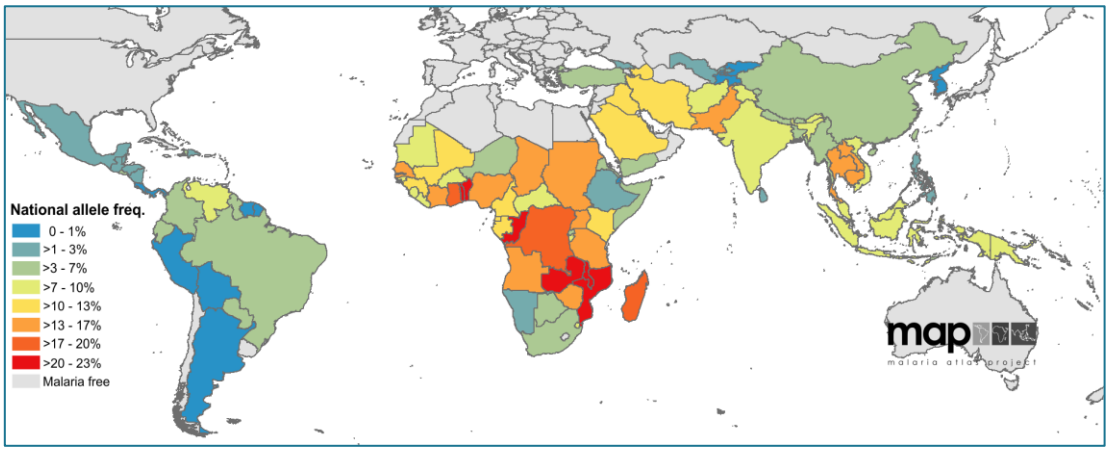
Point-of-care G6PD testing to support safe use of primaquine for the treatment of vivax malaria

WHO Evidence Review Group meeting report
8–9 October 2014, WHO/UNAIDS Building, Geneva, Switzerland

Background

The problem

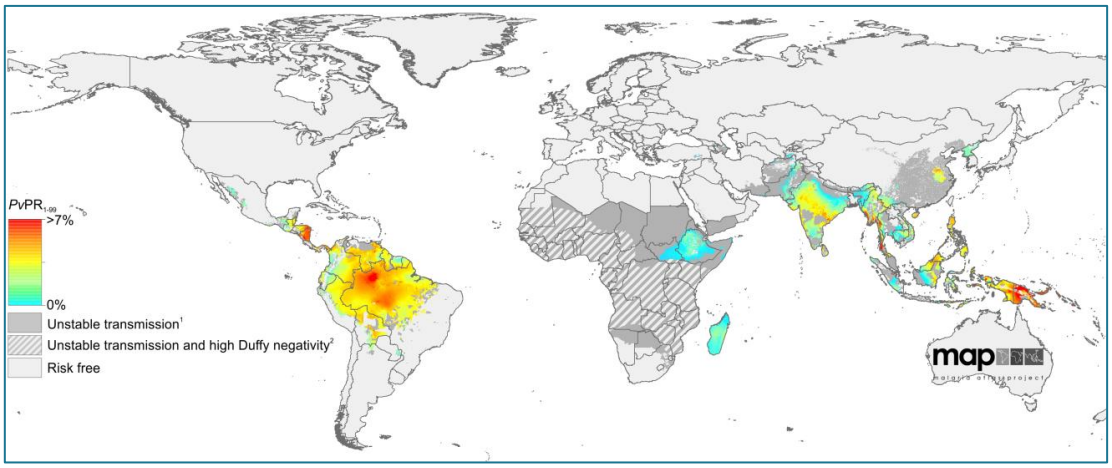
The drug primaquine has been in continuous use since 1952 for the prevention of relapse of *Plasmodium vivax* and *P. ovale*. Primaquine has haemolytic toxicity in those who are deficient in glucose-6-phosphate dehydrogenase (G6PD); also, where most malaria patients live, it is often not practical to test for G6PD deficiency and supervise a 1–2 week course of therapy. Nevertheless, there are no alternative therapeutic options to prevent *P. vivax* relapse (*1*). A daily dose of 0.25 or 0.5 mg/kg for 14 days induces acute haemolytic anaemia (AHA) in patients with inborn deficiency of G6PD (*2*). This genetically heterogeneous X-linked recessive condition affects over 400 million people globally (see Maps 1 and 2 below).



Map 1 National prevalence of G6PD deficiency in males

Across malaria endemic countries, the mean allele frequency of G6PD deficiency is estimated to be 8.0% (interquartile range [IQR]: 7.4–8.8). Since the G6PD gene maps to the X chromosome, the frequency of G6PD deficiency in males is the same as the allele frequency. The frequency of G6PD-deficient females was estimated as the sum of all female homozygotes plus the subset of heterozygotes who have 30% or less of normal G6PD activity (see Table 1). In total, this amounts to about 350 million individuals with G6PD deficiency. Figure reproduced from Howes RE et al. (*3*).

WHO/HTM/GMP/MPAC/2015.6



Map 2 Spatial distribution of Plasmodium vivax malaria endemicity in 2010

The limits of transmission (risk free or unstable annual parasite index [API] <0.1 per 1000 per year)/stable transmission) are defined by API data, supplemented by further data on temperature and aridity masks. Parasite rate population surveys are used in a Bayesian geostatistical framework to model the annual mean endemicity of *P. vivax* across areas of stable transmission, represented in colour and standardized to all ages (1–99 years). Figure reproduced from Gething PW et al. (*4*).

Since 1956, G6PD deficiency has been recognized as the cause of sensitivity to primaquine (*5*). Whenever it is not possible to test for G6PD deficiency before administering primaquine, balanced decision-making is required, weighing the potential risk of harm (i.e. of AHA) upon primaquine intake to a patient who may be G6PD deficient against the risk of harm due to multiple clinical relapses of acute *P. vivax* malaria when primaquine is not administered. Additionally, the contribution of repeated relapses of *P. vivax* infection on morbidity, mortality and transmission needs to be considered (*6, 7*).

Thus, knowing whether a patient is G6PD deficient or not is key to realizing the potential of primaquine anti-relapse therapy to improve *P. vivax* case management and contribute to interruption of transmission. In practical terms, this requires either that the result of a G6PD test done at birth or otherwise is readily available, or that point-of-care (POC) G6PD testing is done following a diagnosis of vivax malaria (*7*). To address this, the WHO Global Malaria Programme, convened an Evidence Review Group (ERG) to examine evidence related to new technologies and devices for testing G6PD deficiency at the POC.

Objectives of the ERG

The specific objectives of this ERG were to apply available evidence in order to answer the following questions:

1. What is the lowest level of prevalence of G6PD deficiency below which there is no need to test for G6PD deficiency before giving primaquine for radical treatment of vivax malaria?
2. What test(s) should be used for classifying males as G6PD normal or deficient?
3. What test(s) should be used in order to identify presumably heterozygous females who are at clinical risk of PQ-induced haemolysis. This requires knowledge on the level of G6PD deficiency (proportion of G6PD deficient red cells, which can vary in heterozygous females between 0% and 100%) at which primaquine will induce a clinically significant haemolysis when given at the standard doses recommended for vivax radical cure.

4. When a male is classified as G6PD deficient, or when a female is classified as a heterozygote with a clinically-relevant proportion of G6PD deficient red cells, or when G6PD heterozygosity cannot be safely diagnosed from female cases, what is the appropriate management of vivax malaria?

    a. No primaquine.
    b. Primaquine at a lower dose but for an extended period.
    c. Primaquine at the standard dose but under medical supervision.

Evidence reviewed

In preparation for the meeting, WHO commissioned a review of the literature on primaquine-related haemolysis risk in G6PD-deficient heterozygous females, kindly completed by Dr J. Recht. In this review, PubMed was searched using the terms "primaquine", "G6PD", "G6PD activity", "G6PD deficiency", "hemolysis" and "heterozygote/ heterozygous". Relevant articles also included those reviewed for a WHO ERG meeting held in 2012 on the safety of gametocytocidal use of primaquine in *P. falciparum* infection (*8*). In addition to published literature identified via PubMed and EmBase searches, studies in press and unpublished evidences were made accessible to the members of the ERG. For G6PD tests, recently published reviews were considered (*9, 10*), providing comparative analysis of the performance of available tests. The evidence reviewed focused on available POC rapid diagnostic tests for G6PD, appropriate for use in tropical and resource-limited settings. A specific qualitative G6PD test (CareStart™) was considered in view of its potential application in resource-limited tropical settings where *P. vivax* is endemic. Also, efforts were made to include unpublished but completed studies evaluating this product, according to a common template that would allow comparable assessment of test performance.

The rapporteurs prepared the present document from several of the pre-reads shared with the participants before the ERG meeting, and from discussions during the meeting. The report was then reviewed in detail by all participants, and any inputs provided were taken into consideration in finalizing the review.

The deliberations of the evidence review group were guided by the knowledge of primaquine-induced toxicity in G6PD-deficient patients, the limitations of historical experience and of published data on safety of primaquine anti-relapse therapy for vivax patients with G6PD deficiency, and the results of recent studies evaluating the performance of qualitative POC G6PD.

Report of the evidence review group

Burden of relapses due to *P. vivax* and health risks

The individual and public health threats posed by relapses due to untreated *P. vivax* liver stage infection need to be taken into account when discussing the risks and benefits of primaquine therapy. The timing and risk of relapse varies widely across geographic regions (see Figure 1 below).

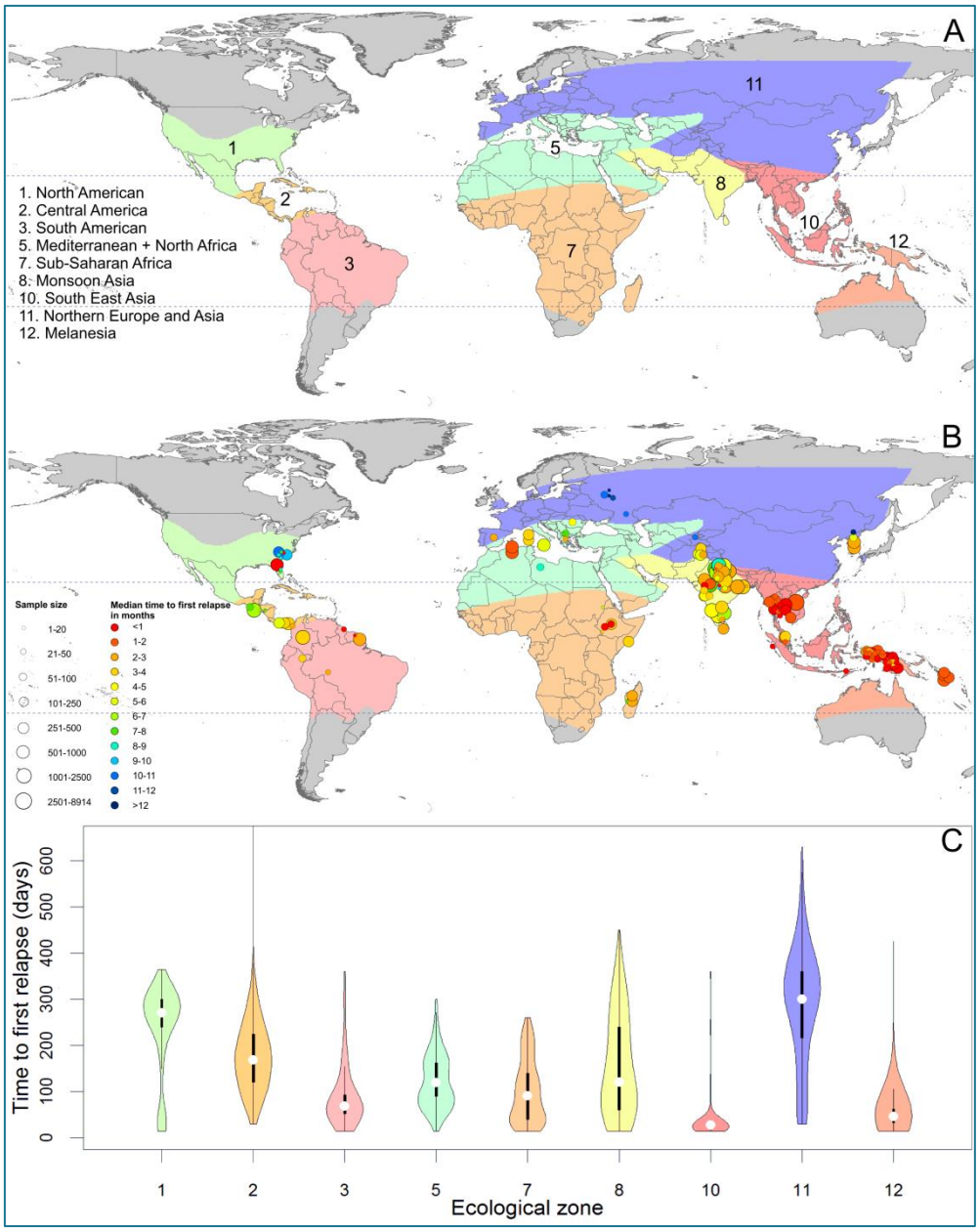


Figure 1 Zones of malaria, surveys used and time to relapse

Map showing ecological zones of malaria (top) and surveys used (middle) to model the time to first relapse in individuals from each zone, illustrated in the graph by violin plots (bottom). From Battle K, et al. (*11*).

Figure 1 above shows that the pattern of rapidly relapsing strains in tropical areas of Latin America and Africa is similar to the relapse behaviour represented by Chesson-like strains of *P. vivax* of tropical Southeast Asia and Oceania. The variation in risk and timing of relapse is key to weighing the specific local risks and benefits of primaquine therapy.

A recent clinical trial of primaquine therapy in Indonesian soldiers provides a clear picture of the burden imposed by vivax malaria not treated with primaquine (*12*). In this study, nearly 80% of the male subjects who did not receive primaquine anti-relapse therapy relapsed within 6 weeks. This represents an incidence of five relapses/person-year, roughly equivalent to the attack rates of *P. falciparum* in high transmission areas of sub-Saharan Africa. This and other studies (*13*)

illustrate the substantial burden of recurrent illness and, by implication, the contribution to vivax transmission by the hypnozoite reservoir, particularly of tropical strains.

Episodes of *P. vivax* malaria, long considered relatively benign, can cause significant and lasting morbidity and even mortality. Recent studies have challenged the dogma that acute vivax malaria is rarely life-threatening (*14-18*). This potential harm must also be considered in the risk–benefit assessment of primaquine therapy. Multiple relapses may contribute substantially to the mortality risks relative to falciparum malaria. In Figure 2, the odds ratio of a fatal outcome among patients hospitalized with a primary diagnosis of vivax malaria relative to falciparum malaria often included 1.0, and the average among them was 0.64 (0.52–0.78).

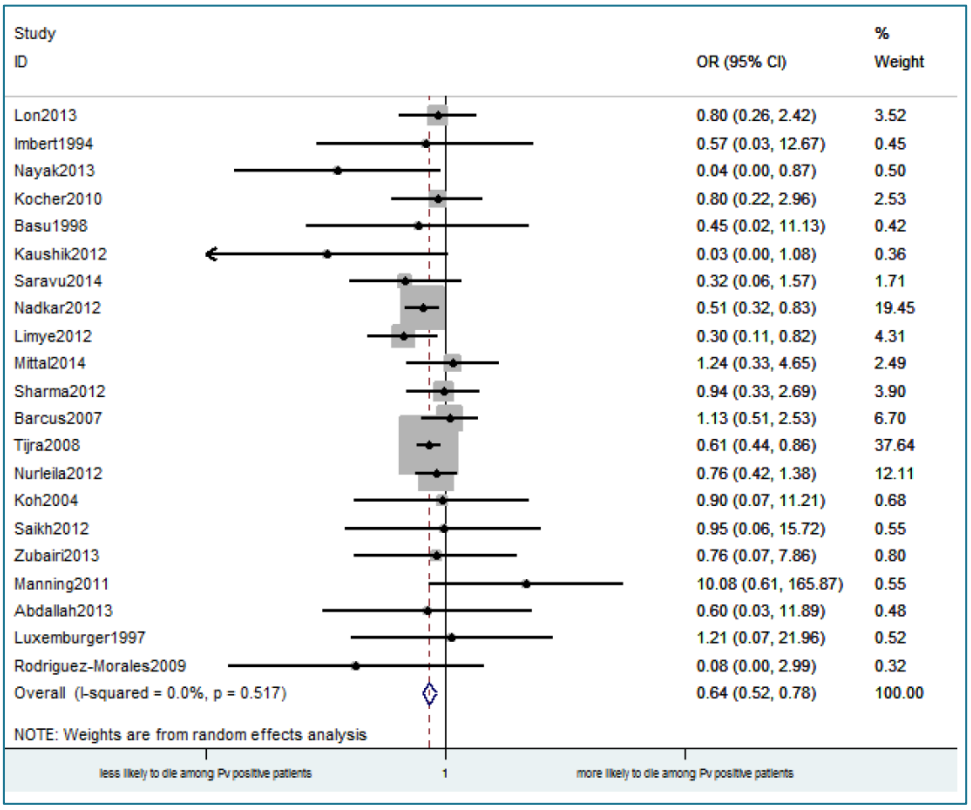


Figure 2 Odds ratio of death as an outcome – comparison of vivax and falciparum malaria

Chart plots the odds ratio (x-axis) of death as an outcome in patients admitted to hospital with a primary diagnosis of vivax malaria relative to the same in patients with a diagnosis of falciparum malaria. An odds ratio of 1.0 represents no difference. Figure reproduced from the WHO Technical briefing on control and elimination of *Plasmodium vivax* malaria (*19*).

These findings serve to underline an important consideration; namely, that although the administration of primaquine in the absence of knowledge of G6PD status can pose serious hazard, so too does withholding primaquine therapy. In settings of high risk of relapse of relatively virulent strains and poor access to good health care, either decision may result in significant consequences. This is the primaquine–G6PD dilemma in the treatment of vivax malaria.

Country policies on primaquine anti-relapse therapy and G6PD testing

Most countries with endemic *P. vivax* recommend anti-relapse therapy with primaquine, in line with WHO recommendations, although four countries (Algeria, Cambodia, Ethiopia and Somalia) do not recommend its use (Figure 4). Most of those countries that do recommend primaquine

recommend the standard 0.25 mg/kg daily dose for 14 days, though some recommend 0.5 mg/kg daily for either 7 or 14 days. A recent meta-analysis suggested efficacy of the 0.25 mg/kg daily dosing regimen (14 days) for temperate strains, and of the 0.5 mg/kg daily dosing regimen (14 days) for tropical frequent relapsing *P. vivax* (*20*). Nonetheless, the programmes of most vivax endemic countries adhere to the lower dose (0.25 mg/kg/day for 14 days) on the basis of the higher risk of more serious harm at the more efficacious higher total dose. Only Iran has adopted the weekly primaquine regimen of 0.75 mg/kg once weekly for 8 weeks without G6PDd testing, as shown in Figure 3.

The application of these recommendations in patients, most with unknown G6PD status, varies among nations, and even within nations among health-care providers. Data on antimalarial tablets suggests that, in some countries where primaquine is recommended without G6PD testing, prescription rates are as low as 10% of vivax cases treated with an antimalarial drug.

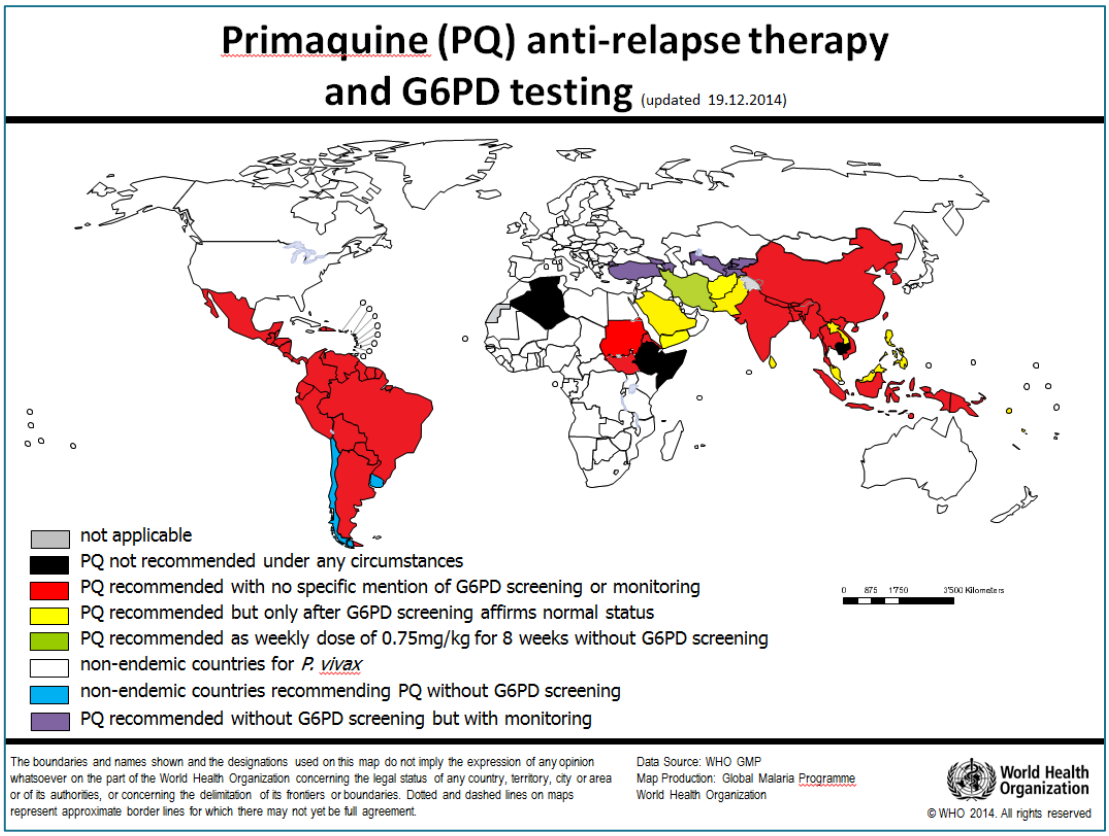


Figure 3 Primaquine therapy as reflected in national treatment guidelines globally
WHO/GMP Survey, 2014 – last updated 19.12.2014.

The reasons for not prescribing primaquine anti-relapse therapy are multifold and complex, but risk of haemolytic toxicity among G6PD-deficient patients is probably the dominant factor. The lack of field-adapted tests, the limited availability of primaquine in many settings and the difficulties in adhering to a 14-day regimen all contribute to what is probably low primaquine effectiveness in many settings.

The problem of female heterozygotes

The ERG considered the implications of G6PD deficiency being an X-linked disorder. This creates two distinct genotypes in males: hemizygous G6PD normal (wild type), and hemizygous G6PD

deficient; and three distinct genotypes in females: homozygous G6PD normal (wild type), and heterozygous and homozygous G6PD deficient (see Table 1). Thus, there are five genotypes in total (two in males and three in females), but only three phenotypes: normal, deficient and intermediate (i.e. from normal to deficient in heterozygous females).

Table 1 Relationship between G6PD genotypes, enzyme activity in red cells, recommended nomenclature and clinical implications with respect to primaquine

Genotype	Sex	G6PD activity	Phenotypic nomenclature	Primaquine sensitivity
XY – wild type	Male	Normal	Normal	No
XX – wild type	Female	Normal	Normal	No
X*Y – hemizygote	Male	<30% of normal	Deficient	Yes
X*X* – homozygote	Female	<30% of normal	Deficient	Yes
X*X – heterozygote	Female	<30% of normal	Deficient	Yes
X*X – heterozygote	Female	Between 30% and 80% of normal	Intermediate	Possible
X*X – heterozygote	Female	>80% of normal	Normal	Unlikely

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.

X chromosome inactivation (or "lyonization", from the name of Mary Lyon who discovered this phenomenon) has major implications in relation to G6PD testing and primaquine toxicity. During embryonic development, one of the two X chromosomes becomes inactivated in every somatic cell in an apparently random manner. However, once inactivation has taken place, the active or inactive state of the X chromosome is faithfully maintained in the progeny of each cell. If a female is heterozygous for a normal and a G6PD deficiency allele, this results in mosaicism: that is, the coexistence of a population of red blood cells (RBCs) that have normal G6PD activity and a population that are G6PD deficient. The relative proportion of deficient to normal RBCs ranges from 0% to 100% in individual heterozygous females, according to a normal (Gaussian) distribution (see Figure 4 below).

This is highly relevant to G6PD testing for the purpose of primaquine therapy. There are two issues:

- *Biological* – In heterozygous females, a cytochemical (flow cytometry or microscopy based) assay of G6PD activity can determine what proportion of RBCs are G6PD deficient; correspondingly, a quantitative assay result will reflect this. At the lower end of the spectrum there will be overlap between heterozygotes and homozygous deficient females; at the upper end of the spectrum there will be overlap between heterozygotes and homozygous normal females (see Figure 5 below).
- *Technical* – Most heterozygotes will have a G6PD activity between 30% and 80% of normal, and in this range the performance of all qualitative screening methods and devices may be ambiguous. In other words, RDTs are mostly meant to give a YES or NO result; however, in heterozygotes, the result may be ambiguous because the enzyme activity is intermediate (see Table 1 above).

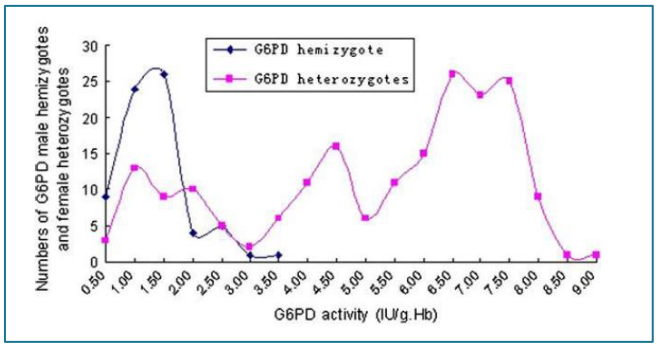


Figure 4 Distribution of male hemizygotes and female heterozygotes for G6PD deficiency along the x-axis of G6PD enzyme activity levels

The males are relatively narrowly distributed at the low end of activity, whereas female heterozygotes appear at all levels of activity. From Jiang WY, et al. (*21*).

In some cases, a rapid diagnostic test (RDT) may classify as normal a heterozygous female who has a substantial proportion of RBCs vulnerable to primaquine-induced haemolysis. This is a conspicuous limitation of RDT (qualitative) G6PD tests, and has been carefully considered in ERG deliberations.

Classification of G6PD deficiency

Variants and degrees of severity

In G6PD-normal patients, primaquine is a remarkably safe and well-tolerated drug with high efficacy in preventing relapses of *P. vivax*. A recent trial of the 0.5 mg/kg primaquine daily dose for 14 days given after therapy with dihydroartemisinin-piperaquine for acute vivax malaria showed good safety and tolerability, with 98% efficacy against relapse (*12*).

Daily primaquine therapy induces AHA in G6PD-deficient patients. The severity of the AHA is variable in relationship to the enzyme half-life and consequent residual level of enzyme activity associated with individual genetic variants of G6PD. Historically, the severity of G6PD deficiency has been classified as mild or severe, largely depending on the reduction in activity of the G6PD enzyme associated with each variant. In the 1980s, WHO adopted a classification scheme that identified common G6PD deficient variants as either class II (activity <10% of normal) or class III (activity >10% of normal) (*22*). The prototype of class II was G6PD Mediterranean and the prototype of class III was G6PD A– (the common African variant). Classification of other variants as class II or class III has been often based on few samples tested in different laboratories. The relationship between enzyme activity and sensitivity to AHA is based on a relatively small number of studies of healthy adult subjects challenged with primaquine.

The physiological difference between African A- and Mediterranean variants may be appreciated from Figure 5.

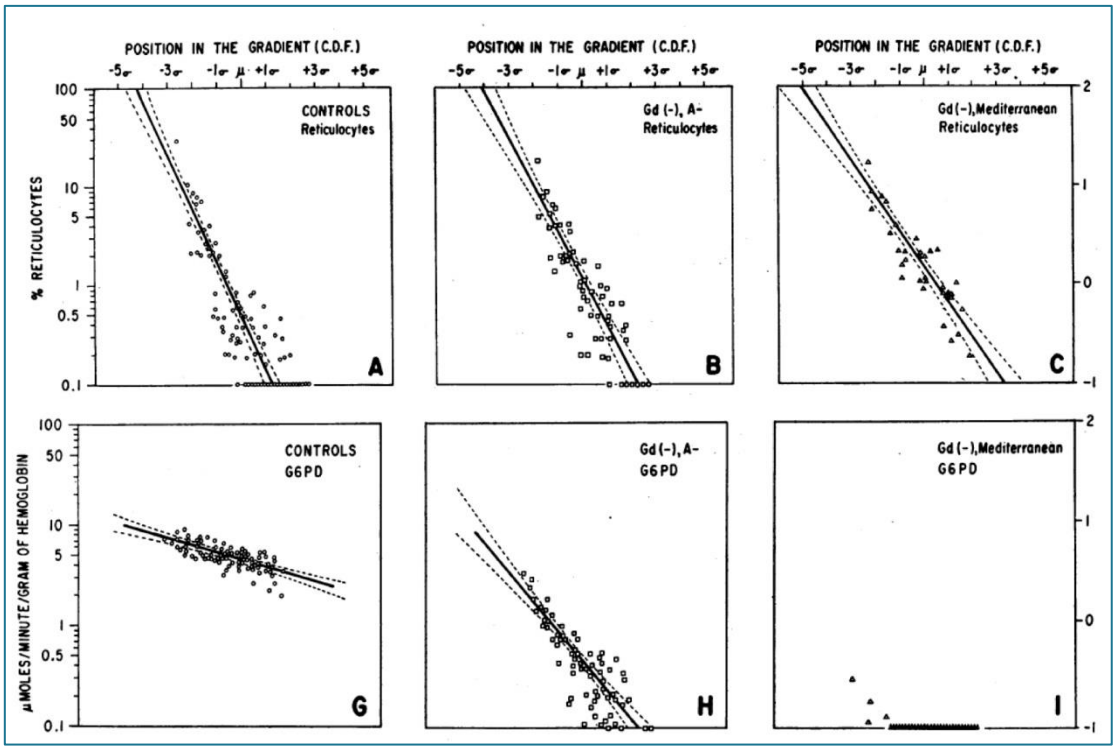


Figure 5 G6PD activity in relation to age of red blood cells

The y-axis (on a log scale) shows G6PD enzymatic activity and the x-axis (linear) the age of RBCs. Upper panels illustrate the proportion of reticulocytes as RBCs age in G6PD normal (panel A), African A– G6PD-deficient (panel B), and Mediterranean G6PD-deficient (panel C) subjects. Lower panels illustrate the decay of G6PD activity as RBCs age in G6PD-normal (panel G), African A– G6PD-deficient (panel H), and Mediterranean G6PD-deficient (panel I) subjects. Taken from Piomelli et al (*23*).

The findings illustrated in Figure 6 concur with the few clinical studies of primaquine challenge in subjects having A– or Mediterranean variants. Those with the A– variant suffer haemolysis of approximately 30% of RBCs within a week of daily doses of 0.5 mg/kg, but thereafter recover to baseline haematocrit, and sustain this level despite ongoing dosing for many weeks (*24*). This compensation is explained by the contingency whereby the oldest RBCs are destroyed and then are replaced by primaquine-tolerant younger RBC populations, which exhibit tolerance even to continued high-dose (30 mg daily) primaquine exposure. In contrast, subjects with the Mediterranean variant do not appear to develop tolerance, because even much younger RBCs (see Figure 6, panel I) have insufficient residual G6PD activity to tolerate the oxidative stress of primaquine. As a result, the haemolytic anaemia is more severe and not self-limiting.

Thus, although there is diversity of G6PD deficiency levels, the risk of AHA upon primaquine therapy applies to all G6PD-deficient variants. The assumption that daily primaquine therapy is relatively safe in patients with the African A– variant is incorrect, because the broadly used characterization of A– variant as “mild and self-limiting” is derived from only a few studies undertaken in small numbers of otherwise healthy male volunteers (*25*). There are three types of G6PD A– in Africa, and it is unknown what the genotype was in subjects included in the primaquine challenge studies undertaken in 1950s and 1960s (although by far the most frequent genotype is that with the G202A nucleotide replacement (*26*)). Indeed, two fatalities in Brazil attributed to primaquine-induced haemolysis, both confirmed at autopsy, carried the A– variant (M. Lacerda, personal communication).

Thus, the classification of variants of G6PD deficiency as “mild” and “severe” for the purpose of guiding clinical decisions should be abandoned. Daily primaquine hyponozoitocidal therapy

(0.5 mg/kg daily for 14 days) induces potentially life-threatening AHA in patients with all of the known G6PD variants, including the A– variant. Further, in view of the limitations of pharmacovigilance in most endemic countries, the relatively small numbers of published reports of serious harm caused by primaquine therapy prescribed without G6PD testing cannot be accepted as evidence of absence of risk of potential harm. In many endemic settings of limited clinical capacities, it seems likely that primaquine-induced AHA may often be inappropriately attributed to the malaria attack or otherwise not understood as being linked to primaquine therapy.

Levels of G6PD activity

The following definitions were adopted in the deliberations of the ERG.

- **In males**: Any male who has red cell G6PD activity less than 30% of the normal mean must be regarded as G6PD deficient (*). It is presumed that he is hemizygous for a G6PD deficiency allele. Any male with a red cell G6PD activity of 30% or more of the normal mean can be regarded as G6PD normal. It is presumed he is hemizygous for a G6PD normal allele.
- **In females**: Any female who has red cell G6PD activity less than 30% of the normal mean or median must be regarded as G6PD deficient. It is presumed that she is either homozygous for a G6PD deficiency allele; or that she has bi-allelic mutations (for instance, one Viangchan allele and one Mahidol allele); or that she is heterozygous for a G6PD deficiency allele, with predominance of a G6PD deficient RBC population. Any female who has a red cell G6PD activity of 80% or more of the normal mean or median can be regarded as G6PD normal. It is presumed that she is either homozygous for a G6PD normal allele; or heterozygous for a G6PD deficiency allele and a G6PD normal allele, with predominance of G6PD normal RBC population. Any female with between 30% and 80% of normal G6PD activity must be regarded as intermediate; it is presumed she is heterozygous for a G6PD deficiency allele and a G6PD normal allele.

(*) – All known G6PD variants that are common or polymorphic have a modal value of red cell G6PD activity that is much less than 30% of normal (for instance, the values are about 5–10% for G6PD A– , <1% for G6PD Mediterranean (*27*) and about <10% for G6PD Mahidol (*28*)). Thus, in calling G6PD deficient any male with an activity <30%, we have preferred to err on the side of caution, considering also the fact that if the test is carried out in a patient who already has a haemolytic condition, the level of enzyme will be increased.

The ERG also discussed the current conventional diagnostic testing terminology. Under those conventions, a G6PD-deficiency screening device would be “positive” for deficiency when G6PD activity is absent, and “negative” for deficiency when G6PD activity is normal. In order to avoid confusion, the ERG agreed that the terminology should be clearer; therefore, test outcomes should be reported not simply as positive or negative, but as “deficient” or “normal”. These terms, and their relation to the definition of G6PD deficiency above with respect to the 30% level of normal enzyme activity, bear directly on how to estimate sensitivity and specificity of G6PD diagnostics. Tests should provide a G6PD “deficient” result for samples with less than 30% normal activity and a “normal” result for samples with greater than 30% normal activity.

Phenotype terminologies are summarized in Table 1, above.

Qualitative tests for G6PD deficiency

Over the past decades, the fluorescent spot test (FST) has been the qualitative recommended screening test for G6PD deficiency (*8*). The ERG accepted that novel qualitative G6PD tests should be at least equivalent to FST in diagnostic performance, because this is the commercial test most widely used by professional clinical laboratory, blood banking, and haematology organizations. However, the procedure is expensive and requires laboratory skills, specialized equipment and a cold chain for labile reagents. These characteristics make the test unfeasible for routine use in rural tropical settings, especially in peripheral health-care facilities where most malaria patients live and seek care (*9*).

More recently, affordable, qualitative POC lateral flow tests that can be performed at or near the site of patient care have become available. These tests generally require whole blood from finger-prick, and can be performed and interpreted by health workers at the bedside or in the field in <30 minutes.

A comparison of the diagnostic performance of available qualitative tests for G6PD deficiency based on published studies is shown in Table 2, below.

Table 2 Diagnostic performance of qualitative tests for G6PD deficiency

Ref	test	specimen	Gold Std	Threshold	# samples	# deficients	heterozygotes	Sensitivity %
1	WST8 /1-methoxy PMS	Finger prick /DBS	R and D diagnostics	<60% median of males and females	235	30 (all > 10% normal)		72
2	FST	venous	Trinity	Median of normal males	214	23	25	100 (30%) 91 (60%)
3	FST	venous	BIOLABO SA/	10% genotype	295	42	34	100 (10%) 43 (genotype)
4	*FST*	*?*	*Geno-type*	*All normal by FST*	*461*	*27*	*61*	*All misclassified by FST*
5	Binax NOW	venous	Trinity	4.0 U/gHb	246	50	-	98
6	Binax NOW	venous	Trinity	<60% median of males and females	356	11	-	54.5
2	Binax NOW	venous	Trinity	Median of normal males	214	23	25	100 (30%) 83 (60%)
7	1st gen Care Start	venous	Trinity	Lower limit from 174 normal subject (~30%	903	97	-	68
8	Care Start	venous	Trinity	Mean from >4.56 IU/gHb and [Hb] >12 g/Dl	456	46(<30%)	-	90 (<10%) 84.8 (<30%)

[1]de Niz et al. 2013 (*29*), [2]la Rue et al. 2014 (*30*), [3]Nantakomol et al. 2013 (*31*), [4]Eziefula et al. 2014 (*32*), [5]Tinley et al. 2010 (*33*), [6]Osorio et al. 2014 (*34*), [7]Kim et al., 2011 (*35*) and [8]von Fricken et al. 2014 (*36*). Superscripts denote number in table.

Diagnostic performance of CareStartTM G6PD test

Due to the limitations of the FST and the temperature restrictions for use of the FDA-approved Binax Now G6PD test, the evidence review focused on independent evaluations (published and unpublished) of the only commercially available test potentially appropriate for use in tropical *P. vivax* endemic settings, namely CareStart™ G6PD RDT (Access Bio Inc.).a A common template that would allow comparable assessment of CareStart G6DP RDT performance across unpublished studies was complete by the principal investigators and key study characteristics and results are presented in Tables 4 and 5 (*29*).

The published studies of the CareStart™ G6PD test available at the time of the meeting were encouraging particularly findings of von Fricken et al. in which the assay had 90% sensitivity for detecting subjects with <10% G6PD activity. However, sensitivity reduced to 84.8% for patients with <30% G6PD activity (*36*).

Data from unpublished studies evaluating an improved version of the CareStart G6PD RDT compared to both quantitative assays and different brands of FST, produced more encouraging results with laboratory technicians performing the tests in laboratory settings (only one study was performed by lab technicians in the field, Satyagraha ref. #4, in Table 3 and 4).

a. Access Bio has recently launched and CE marked a quantitative Biosensor product, but no published or unpublished reports of independent evaluations were available to review (*30, 31, 33*).

Table 3 Assessment of different commercially available G6PD diagnostic screening tests in male subjects from different countries

Study/PI	Test	Sample Type	Setting	Operator	Reader Assessment	Temp (^{0}C)	Sensitivity (%)/CI	Specificity (%)/CI	PPV (%)	NPV (%)	Prevalence (%)/ Sample Size	Reference Standard
Cambodia/ D. Menard*[1]	CareStart v2	Venous & Capillary	Mobile lab	technician	2 independent readers, if discordant, a third reader	26–29	100.0	98.7	92.2	100.0	15.0/392	G6PD Quantitative Trinity Biotech
Thailand/ G. Bancone*[2]	CareStart v2	Venous	Lab	technician	2 independent readers, if discordant, a third reader	28–29	87.5	100.0	100.0	89.7	9–18/150	G6PD Quantitative Trinity Biotech
		Capillary					100.0	100.0	100.0	100.0		
Thailand/ G. Bancone*[2]	R&D Diagnostic	Venous	Lab	technician		28–29	96.0	100.0	100.0	96.3	9–18/150	
		Capillary					100.0	100.0	100.0	100.0		
Indonesia/A. Satyagraha*[3]	CareStart v2	Venous	Field	technician	1 reader, if unsure, another reader	29–34	100.0/ (100.0–100.0)	98.7/ (97.3–100.0)	89.0/ (77.0–100.0)	100.0/ (100.0–100.0)	9.2/260	G6PD Quantitative Trinity Biotech
Indonesia/A. Satyagraha*[3]	FST Trinity Biotech	Venous in EDTA	Lab	technician	2 readers, if discordant, a third reader	26–29	91.7 / (80.6–100.0)	92.4/ (89.0–95.8)	55.0/ (40.0–70.0)	100.0/ (100.0–100.0)	8.5/260	G6PD Quantitative Trinity Biotech
Brazil/M. VG Lacerda*[4]	CareStart	Venous in EDTA	Lab	technician	2 readers, if discordant, a third reader	19–26	61.5	98.3	42.1	99.2	1.9/674	G6PD Quantitative Pointe Scientific

* Based on the result of 30% cut-off value of normal G6PD activities
[1] Based on Roca-Feltrer et al. (*37*)
[2] Based on Bancone et al. (*28*)
[3] The G6PD prevalence was based on the FST or RDT results against the reference standard result.
[4] Study in males only.

Table 4 Assessment of different commercially available G6PD diagnostic screening tests in female subjects from different countries

Study/PI	Test	Sample Type	Setting	Operator	Reader Assessment	Temp (^{0}C)	Sensitivity (%)/CI	Specificity (%)/CI	PPV (%)	NPV (%)	Prevalence/ Sample Size	Reference Standard
Cambodia/ D. Menard*[1]	CareStart v2	Venous & Capillary	Mobile lab	technician	2 independent readers, if discordant, a third reader	26–29	100.0	94.5	36.6	100.0	3.6/419	G6PD Quantitative Trinity Biotech
Thailand/ G. Bancone	CareStart v2	Venous	Lab	technician	2 independent readers, if discordant, a third reader	28–29	90.9	97.4	90.0	97.4	-	G6PD Quantitative Trinity Biotech
		Capillary					100.0	82.7	60.6	100.0		
Thailand/ G. Bancone	R&D Diagnostic	Venous	Lab	technician		28–29	95.5	97.4	91.3	98.7	-	
		Capillary					100.0	97.4	91.7	100.0		
Indonesia/A. Satyagraha*[2]	CareStart v2	Venous	Field	technician	1 reader, if unsure, another reader	29–34	83.3/ (53.5–100.0)	92.7/ (90.0–95.5)	17.0/ (3.0–30.0)	100.0/ (99.0–100.0)	1.4/350	G6PD Quantitative Trinity Biotech
Indonesia/A. Satyagraha*[2]	FST Trinity Biotech	Venous in EDTA	Lab	technician	2 readers, if discordant, a third reader	26–29	100.0/ (100.0–100.0)	92.2/ (89.3–95.0)	18.0/ (5.0–31.0)	100.0/ (100.0–100.0)	1.7/350	G6PD Quantitative Trinity Biotech

* Based on the result of 30% cut-off value of normal G6PD activities.
[1] Based from Roca-Feltrer et al. (*37*)
[2] The G6PD prevalence was based on the FST or RDT results against the reference standard result.

Preferred product characteristics of qualitative point-of-care G6PD RDTs

Review of the performance data for CareStart G6PD RDT prompted discussion on preferred product characteristics for POC G6PD RDTs. Specifically, there was consensus for the following product characteristics:

- → >95% sensitive compared to spectrophotometry or equivalent quantitative tests at detecting G6PD enzyme activity levels <30% of normal;
- → negative predictive value of >95%, 95% probability that the patient has >30% normal G6PD activity, when the diagnostic test yields a non-deficient result;
- → stable at temperatures expected in tropical settings (30–40°C); and
- → visual readout that clearly distinguishes between "deficient" and "normal" patients.

G6PD testing and female heterozygotes

As already mentioned, classification of a heterozygote female as G6PD deficient is not straightforward, because the majority of heterozygotes have intermediate levels of G6PD activity. In other words, a qualitative classification of "normal" will include many heterozygotes with intermediate G6PD activity. In light of this issue Baird et al. (*38*) evaluated diagnostic performance of the FST and CareStartTM G6PD kits using an in vitro copper G6PD inhibition model. The graph in Figure 6 below illustrates the key findings.

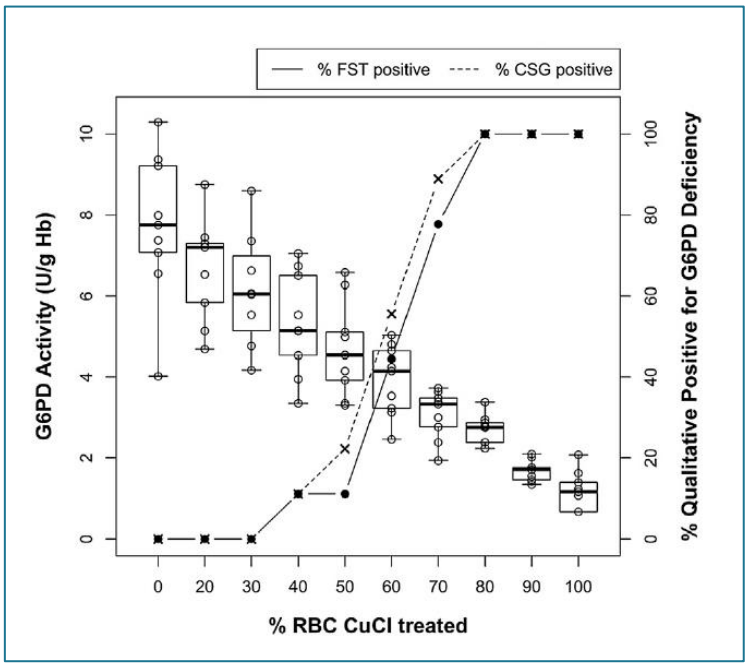


Figure 6 Relationship between net residual enzyme activity in red blood cell suspensions containing increasing proportions of wholly-deficient (copper treated) RBC, along with diagnostic performance of the FST and CareStartTM G6PD kits

From Baird et al. (*38*)

These data confirm that as the proportion of normal RBCs rises from 30% to 80%, the ability of either kit to result in a "normal" classification increases linearly without a clear cut-off point. In general, at 50% normal RBCs, the probability of being classified as deficient is about 50%. The probability of a "normal" classification increases linearly with increasing net G6PD activity.

Consequences of primaquine dosing in G6PD heterozygotes

Daily dosing for 14 days (at 0.25 mg/kg)

There are few studies evaluating the haematological effects of primaquine in heterozygous G6PD deficient females. Only two published studies (*39, 40*) reported hemolytic effect of primaquine given to heterozygous females for 14 days as part of a vivax malaria treatment regimen. In one of the studies (*40*), the subjects were mostly of G6PD Mahidol genotype. In four studies that included heterozygous females with the G6PD A- variant, subjects were administered a single dose of primaquine and the Hb response followed. A heterozygous child with severe anaemia recovered well without requiring blood transfusion. In the other study [*39*] with a small sample size, the fall in Hb observed by day 7 post-primaquine administration was similar among heterozygous females compared to that among hemi/homozygotes given higher primaquine doses.

In a GSK-sponsored study of tafenoquine (TAF 110027), 4 heterozygous women were treated with 15 mg primaquine base for 14 days and showed a pattern and level of drop of Hb (2.5 g/dL) similar to that observed in all patients with G6PD deficiency (Figure 7 below shows individual haematological parameters). These women had G6PD activity levels ranging between 40% and 60% of normal (J. Green, personal communication).

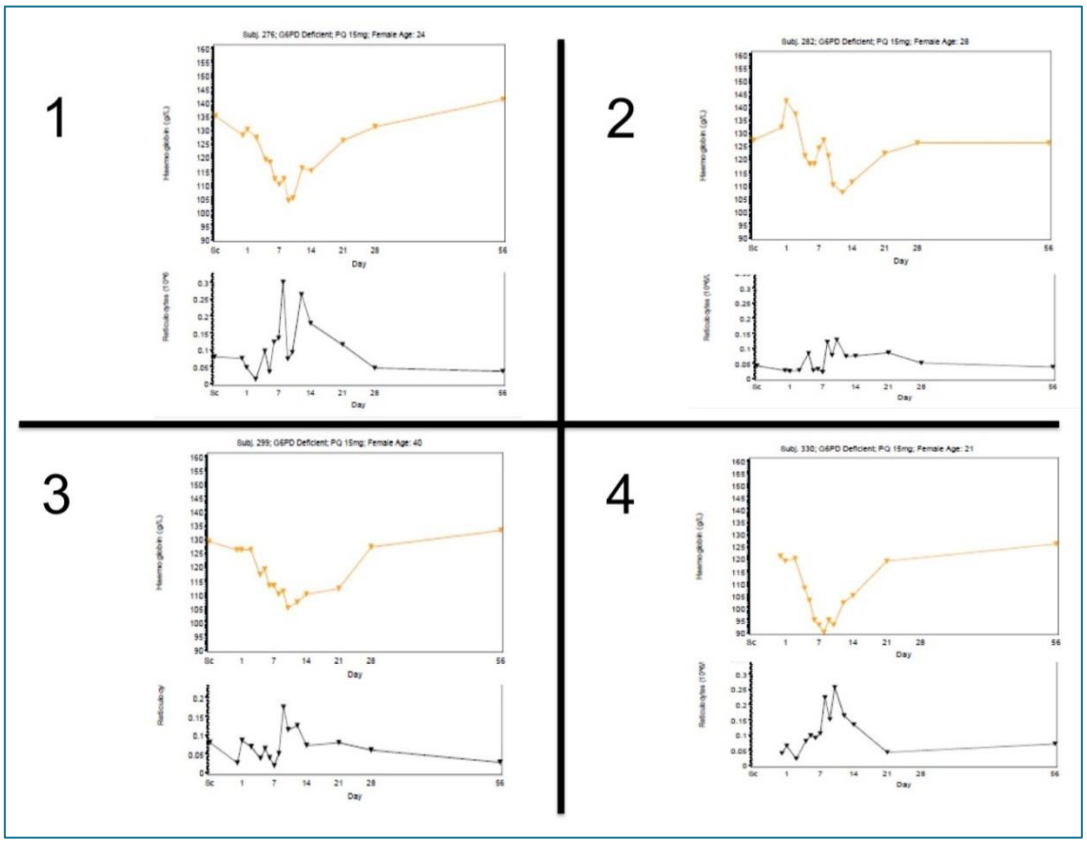


Figure 7 Haemoglobin (orange, above) and reticulocyte (black, below) levels with daily primaquine for 14 days at 0.25 mg/kg/day among four women heterozygous for G6PD deficiency

Courtesy of GSK.

The above figures demonstrate a substantial hemolytic response to daily primaquine at 0.25 mg/kg in heterozygous females with G6PD deficiency. The haemoglobin nadir occurs

between days 8 and 11, representing drops in haemoglobin ranging from 17% to 25%; thereafter the haemoglobin recovers to pretreatment levels despite continued primaquine dosing up to day 14. As shown in all graphs, the spike in reticulocytosis following the AHA commences at about day 7 of dosing. These findings, in Mahidol variant women having 40% to 60% of normal G6PD activity effectively mirror those in the experiments shown in Figure 8 below, African A - hemizygous men.

No firm conclusions may be drawn regarding extent of hemolysis and risk in broader human populations on the basis of only four subjects. Nonetheless the experiments affirm that female heterozygotes of the "deficient" phenotype (<30% of normal G6PD) will certainly hemolyse following exposure to daily primaquine therapy, even at the lower 0.25 mg/kg dose.

Weekly dosing for 8 weeks (at 0.75 mg/kg)

The ERG considered recent evidence concerning the safety of repeated weekly dose of 45 mg primaquine (0.75 mg/kg) for 8 weeks. This regimen has been recommended by WHO since soon after its description in 1960 as a safe and efficacious regimen. However, it is based on in relatively small number of otherwise healthy volunteers carrying the less severe G6PD A- variant (*24*). Figure 8 below summarizes some of those findings.

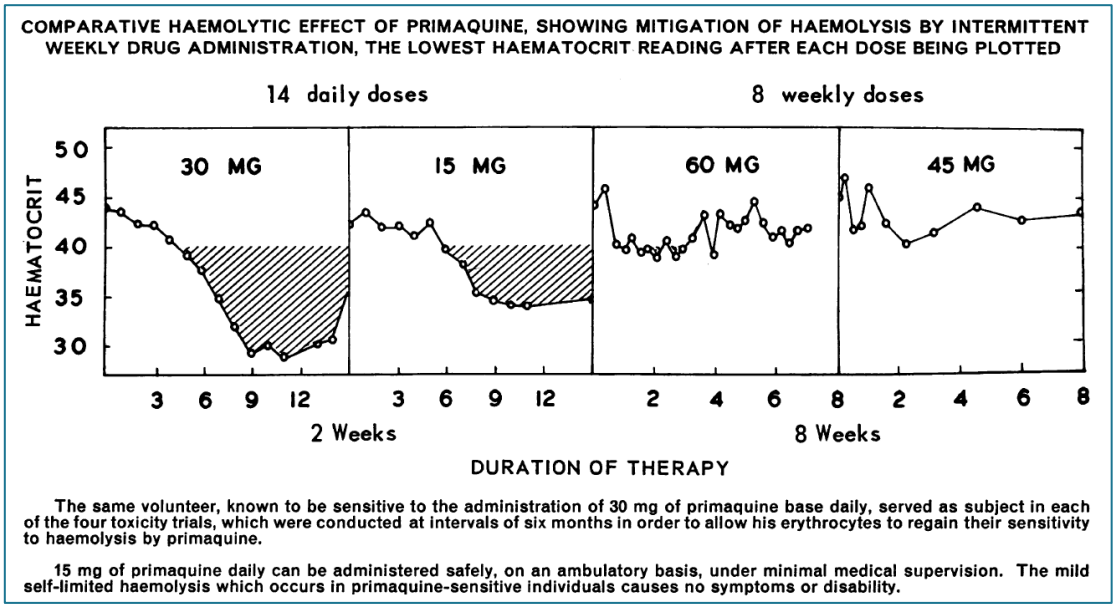


Figure 8 Distinction in haemolytic response between daily versus weekly dosing with primaquine in the same human subject represented by each of the four panels

Reproduced rom Alving et al. (*24*).

In a recent study in Cambodia, the safety of the 0.75 mg/kg weekly dose of primaquine was assessed in hemizygous males carrying the Viangchan variant of G6PD deficiency and suffering acute vivax malaria (Kheng Sim et al., submitted). A total of 75 patients were enrolled aged between five to 63 years. G6PD status was normal in 57 patients and deficient in 18: Canton (n=1) and Viangchan (n=17). Three of the latter were heterozygous females and all other deficient patients were hemizygous males. The variation of Hb concentration over time after treatment with 45 mg primaquine base on a weekly basis is shown in the Figure 9 below.

There was extensive debate on the risk associated with a high dose regimen (even with weekly dosage), and how this can only be safely administered with close monitoring and facilities with

blood transfusion capabilities. Haemoglobin levels in both deficient and normal subjects declined in the two days following therapy, and only slightly more steeply among the deficients. Haemoglobin concentration then levelled and gradually increased, despite doses given again on days 14, 21, 28, 35, 42, and 49 of the study.

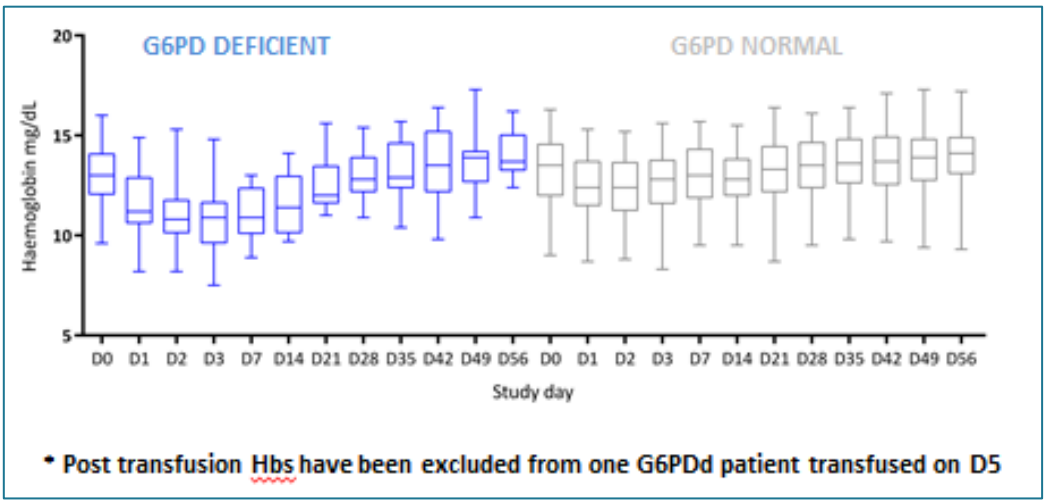


Figure 9 Graphs show responses to single weekly doses of 45 mg primaquine in 18 G6PD deficient (left) and 56 normal (right) male patients with acute P. vivax in western Cambodia

Recommendations on G6PD testing and primaquine anti-relapse therapy

After reviewing the evidence presented, and considering the specific questions listed as specific objectives of the meeting, the ERG elaborated the following recommendations for consideration by the Malaria Policy Advisory Committee.

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.
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.
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.
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.
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 0.75 mg/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.
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
7. Patients diagnosed with acute P. vivax or P. ovale malaria and whose G6PD status is unknown may receive a weekly dose of 0.75 mg/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.

Annex 1 – Listing of the meeting pre-reads

1. Baird, JK *et al*. Noninferiority of Glucose-6-Phosphate Dehydrogenase Deficiency Diagnosis by Point-of-care Rapid Test vs the Laboratory Fluorecent Spot Test Demonstrated by Copper Inhibition in Normal Human Red Blood Cells. (2014) *Translational Research, online September.*

2. LaRue, N *et al*. Comparison of quantitative and qualitative tests for glucose-6-phosphate dehydrogenase deficiency (2014). *Am. J. Trop. Med. Hyg. 91*: 854–861.

3. Luzzatto, L & Seneca, E. G6PD Deficiency: a classic example of pharmacogenetics with on-going-going clinical implications (2014). *BJH 164*:469–480.

4. Osorio, L *et al*. Performance of BinaxNOW G6PD deficiency point-of-care diagnostic in P. vivax-infected subjects (2014). *Am. J. Trop. Med. Hyg.* (accepted).

5. Bancone, G. Diagnostic performance of G6PD POC tests in Thailand.

6. Domingo, G. Overview of performance of point-of-care tests of G6PD deficiency available on the market.

7. Douglas, T. Is it ever ethically acceptable to use primaquine as a hypnozoitocide for vivax malaria without testing for G6PD deficiency?

8. Green, J. Clinical summary of investigations to determine the hemolytic potential of Tafenoquine in G6PD deficient subjects.

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

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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.

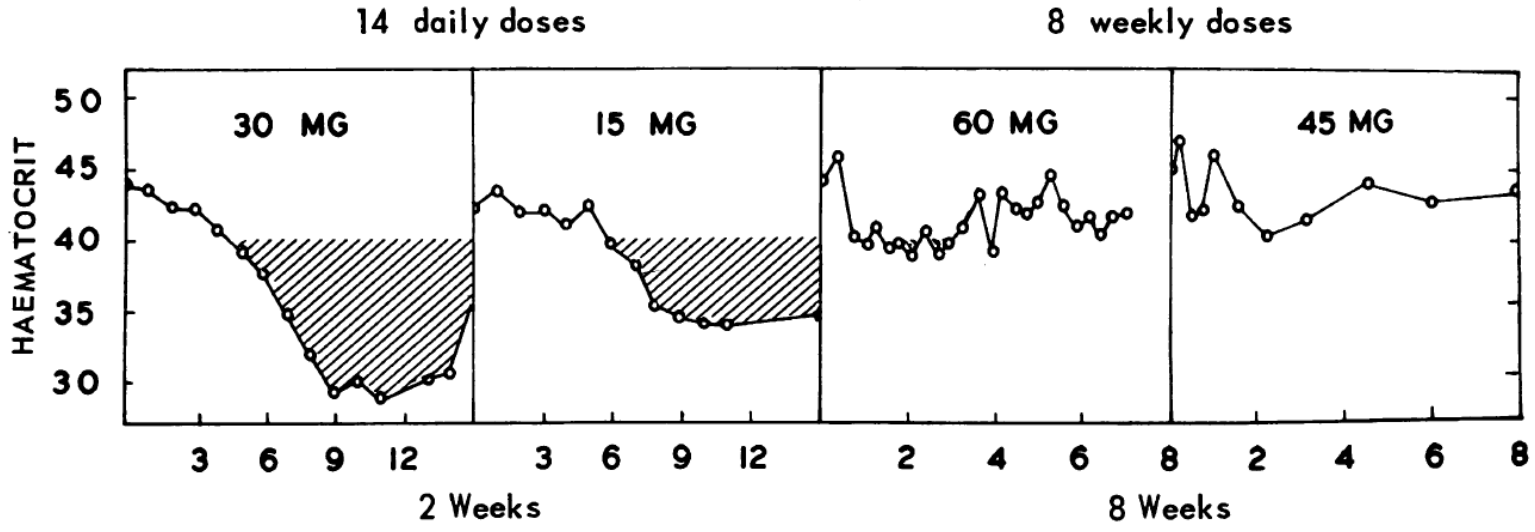
Why an ERG in 2014?!

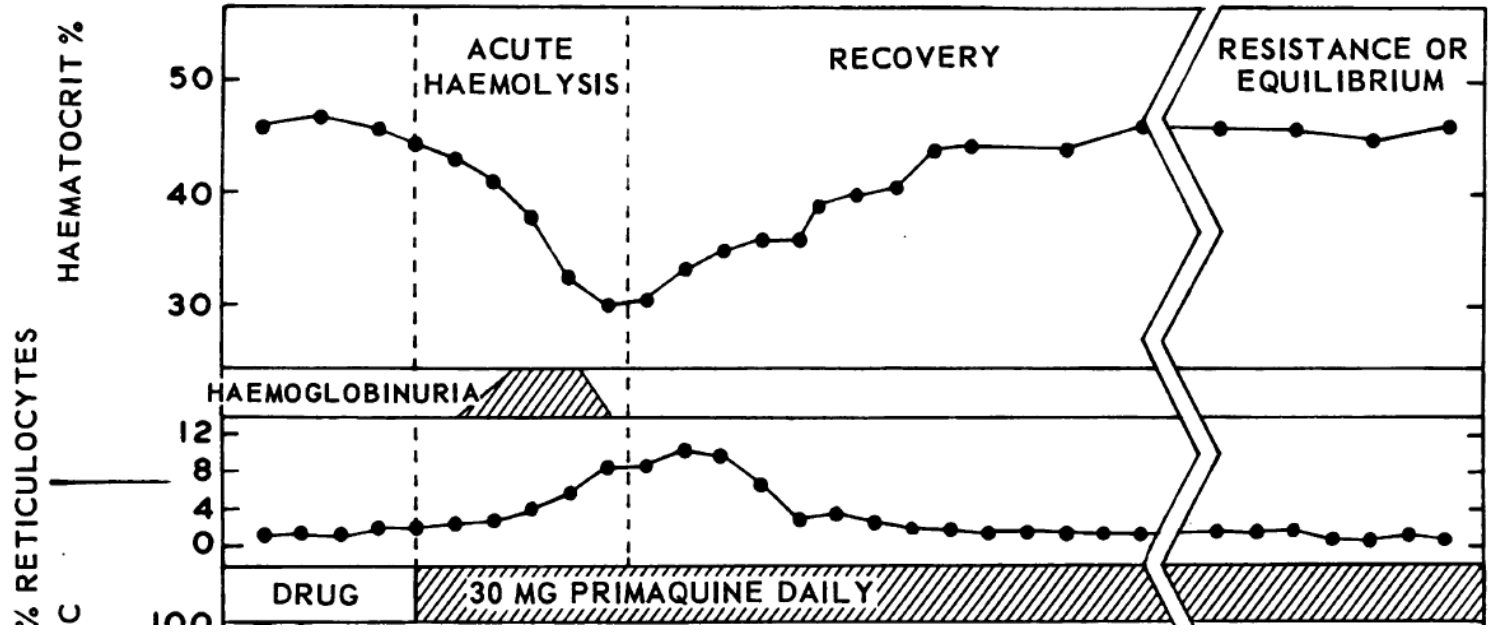
- Primaquine registered for anti-relapse Rx in 1952
- G6PD deficiency discovered in 1956
- US Army invented primaquine and used it before G6PD deficiency was even known – so who needs screening?
- US Army experience based on mild, self-limiting A- variant, and they had very few poor outcomes (Korean 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

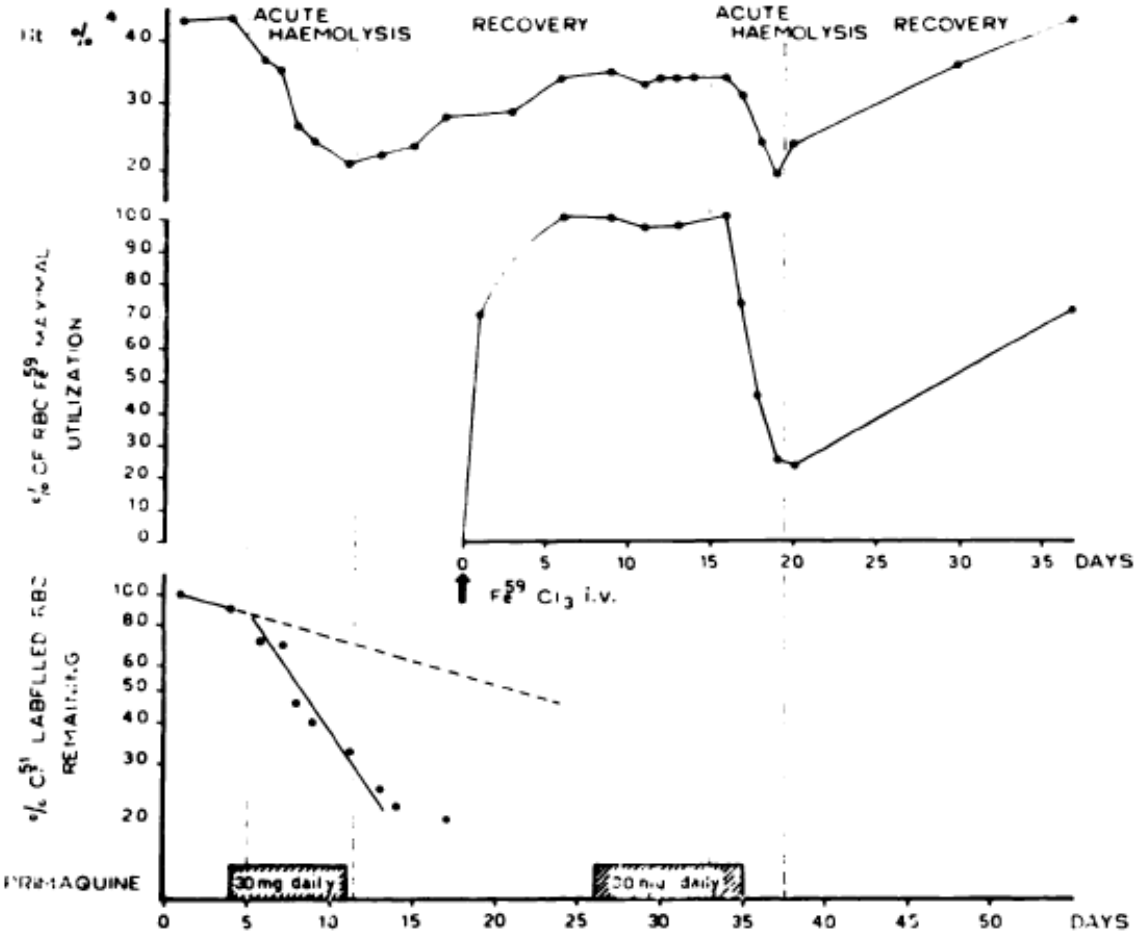
U.S. Army view of G6PD deficiency



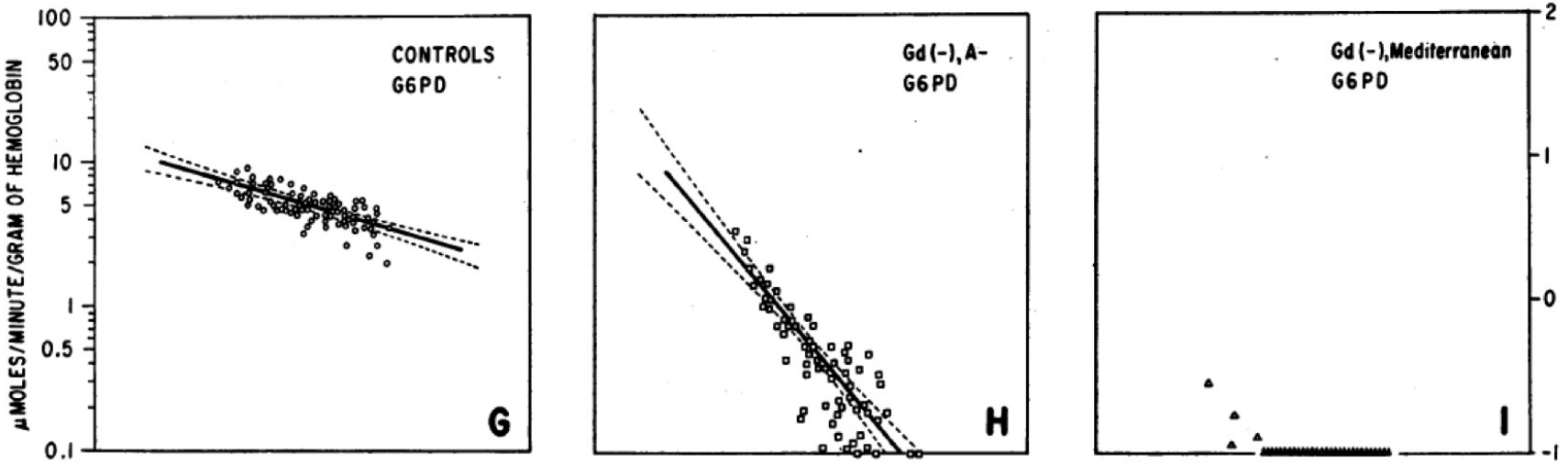


Alving et al.,
Bull WHO 1960

Discovering variable G6PD deficiency



Variable G6PD deficiency



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

Sergio Piomelli, Laurence M. Corash, Deatra D. Davenport, Janet Miraglia, and Edward L. Amorosi

But rationalizations continued

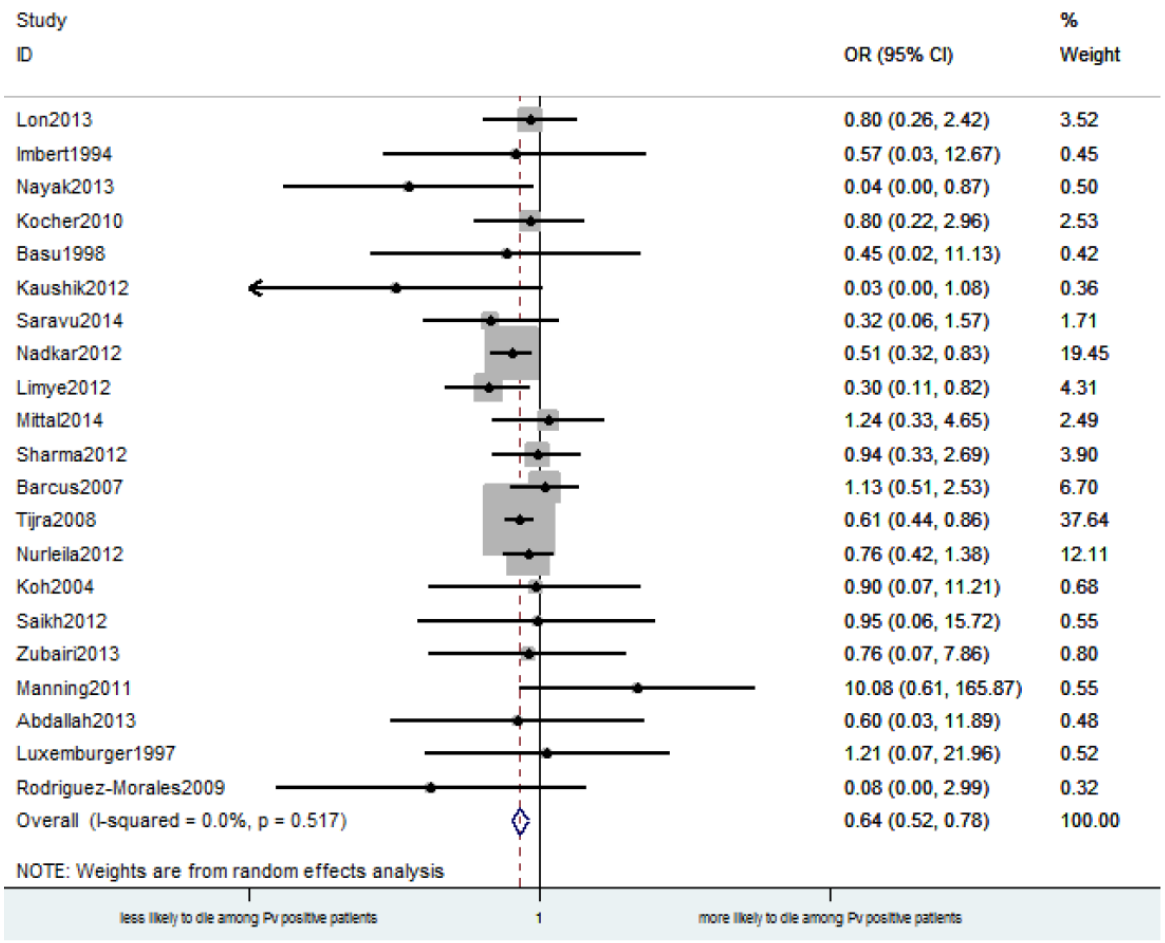
- "*Reports on large numbers of patients treated with this regimen, even where G6PD deficiency is quite common, indicate this regimen is generally well tolerated and that hemolyisis, when it occurs, is mild and self-limiting.*" WHO TGM 1981
  - "*No harm done in using primaquine without G6PD screening*"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

World Health Organization

Plasmodium vivax Malaria

Technical Briefing

Draft 14th August

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.

Primaquine dilemma acknowledged

World Health Organization

Plasmodium vivax Malaria

Technical Briefing

Draft 14th August

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

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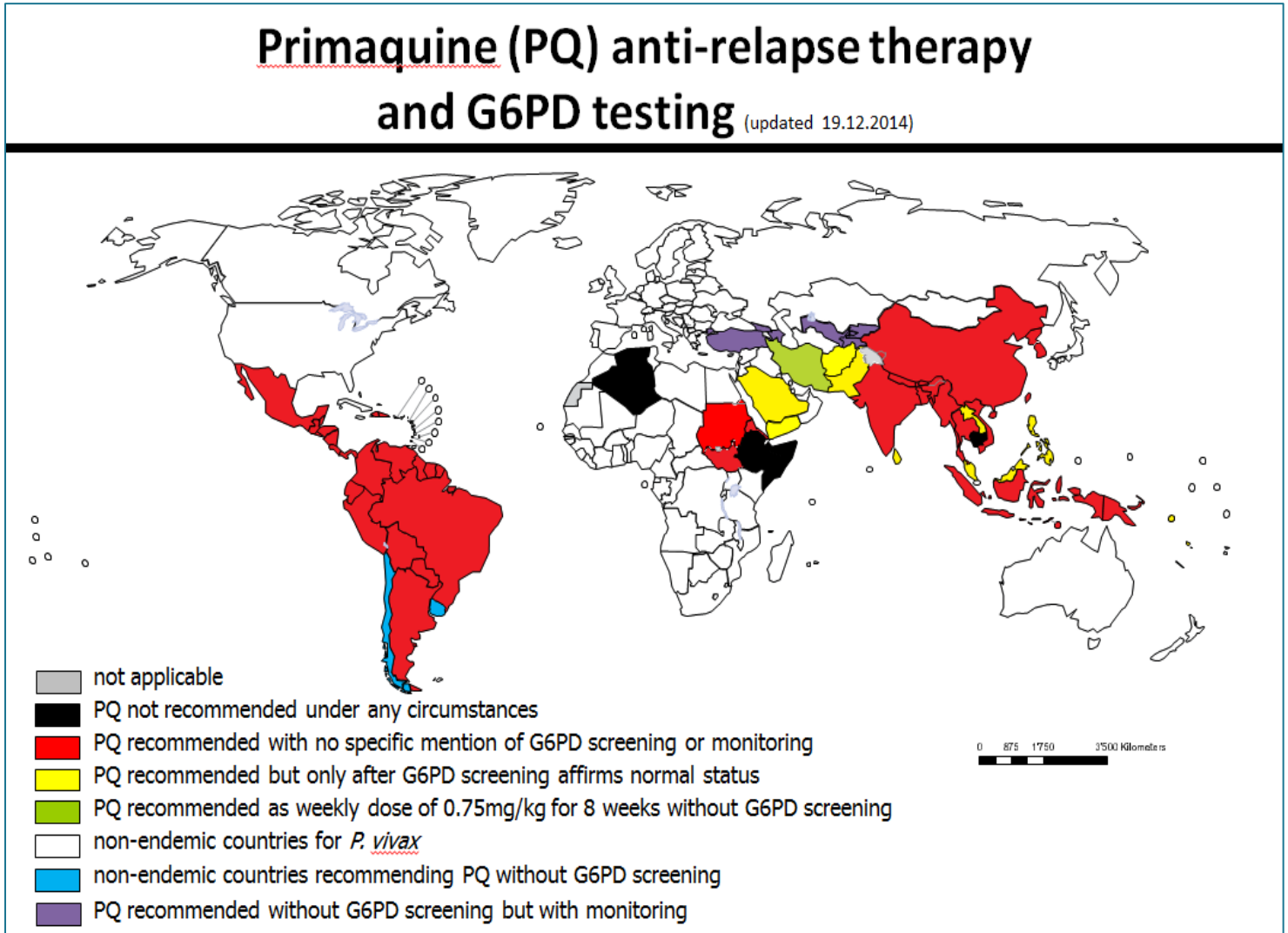
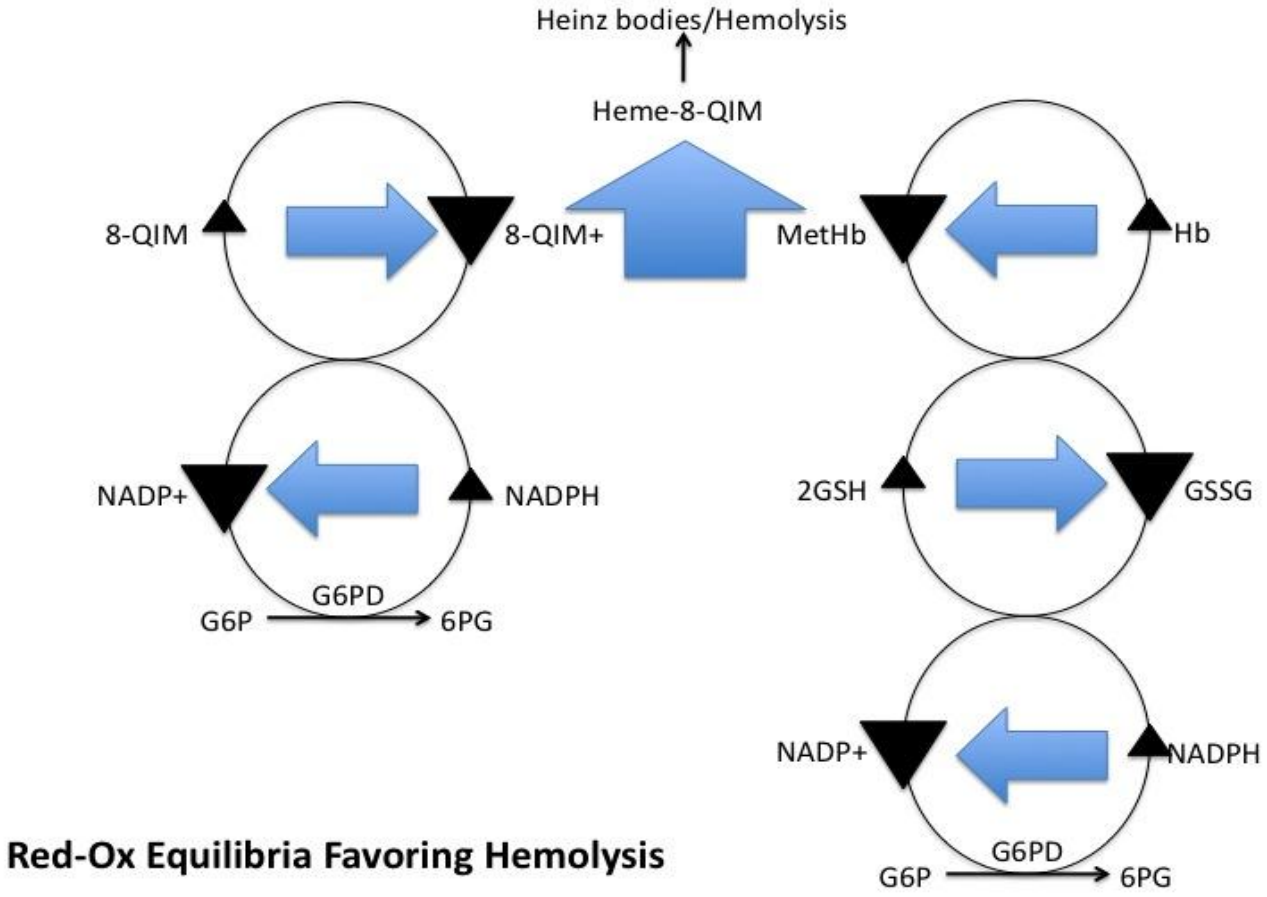
Primaquine practice



G6PD biology



Red-Ox Equilibria Favoring Hemolysis

G6PD biology

Genotype	Sex	G6PD activity	Phenotypic nomenclature	Primaquine sensitivity
XY – wild type	Male	Normal	Normal	No
XX – wild type	Female	Normal	Normal	No
X*Y – hemizygote	Male	<30% of normal	Deficient	Yes
X*X* – homozygote	Female	<30% of normal	Deficient	Yes
X*X – heterozygote	Female	<30% of normal	Deficient	Yes
X*X – heterozygote	Female	Between 30% and 80% of normal	Intermediate	Possible
X*X – heterozygote	Female	>80% of normal	Normal	Unlikely

KEY ISSUE

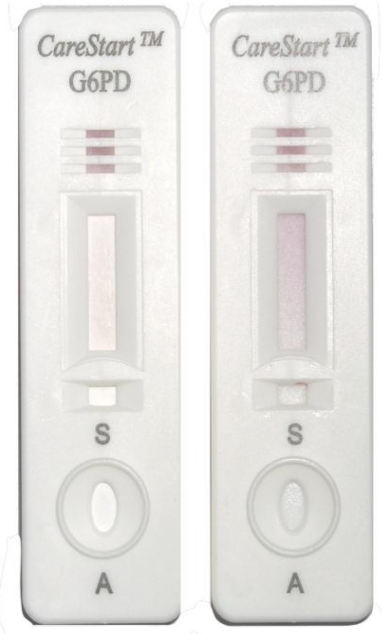
NB – The classification of "intermediate" is possible with quantitative testing but does not yet inform the decision of whether to proceed with primaquine therapy or withhold it. IN QUALITATIVE TEST, "INTERMEDIATE" WILL BE CLASSIFIED AS "NORMAL"

G6PD diagnostics

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

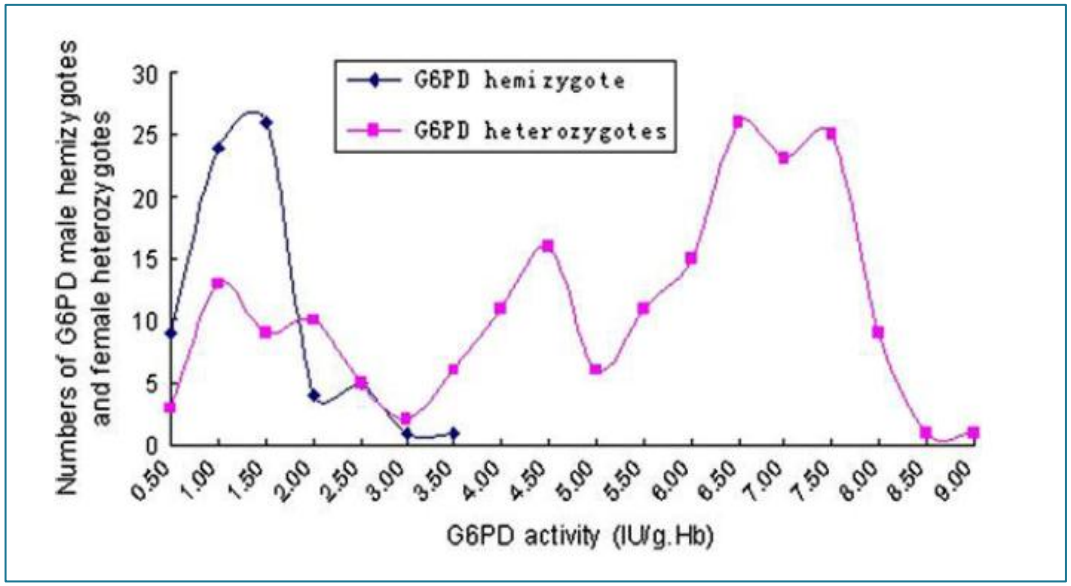
Point of care G6PD diagnostics

• No laboratory skills	***Yes***	***Yes***
• No laboratory equipment	***Yes***	***Yes***
• No cold chain	***Yes***	***Yes***
• Ambient temperature use	***Yes***	***No***
• Low cost	***Yes***	***No***

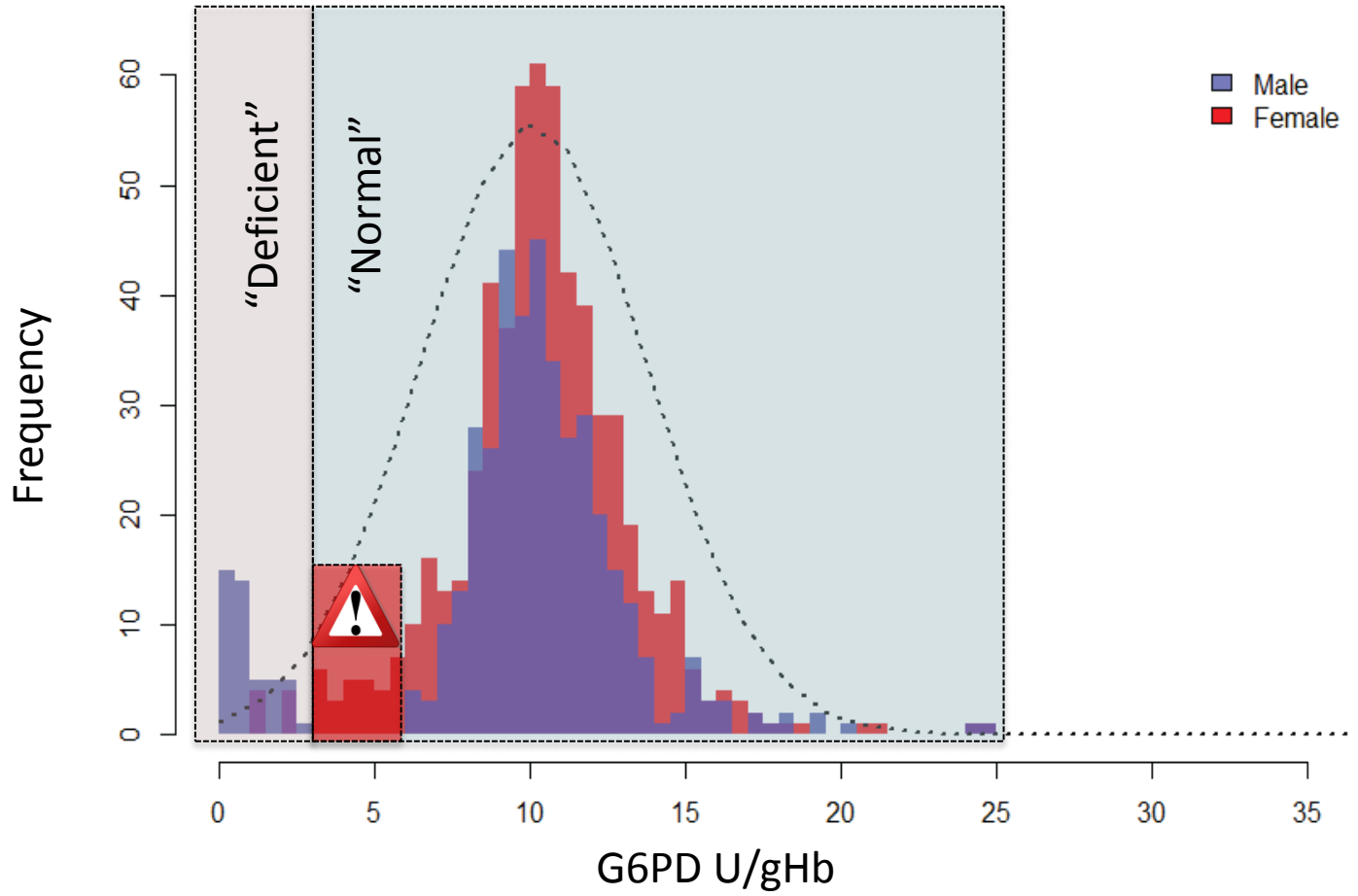


Heterozygous females & G6PD screening

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

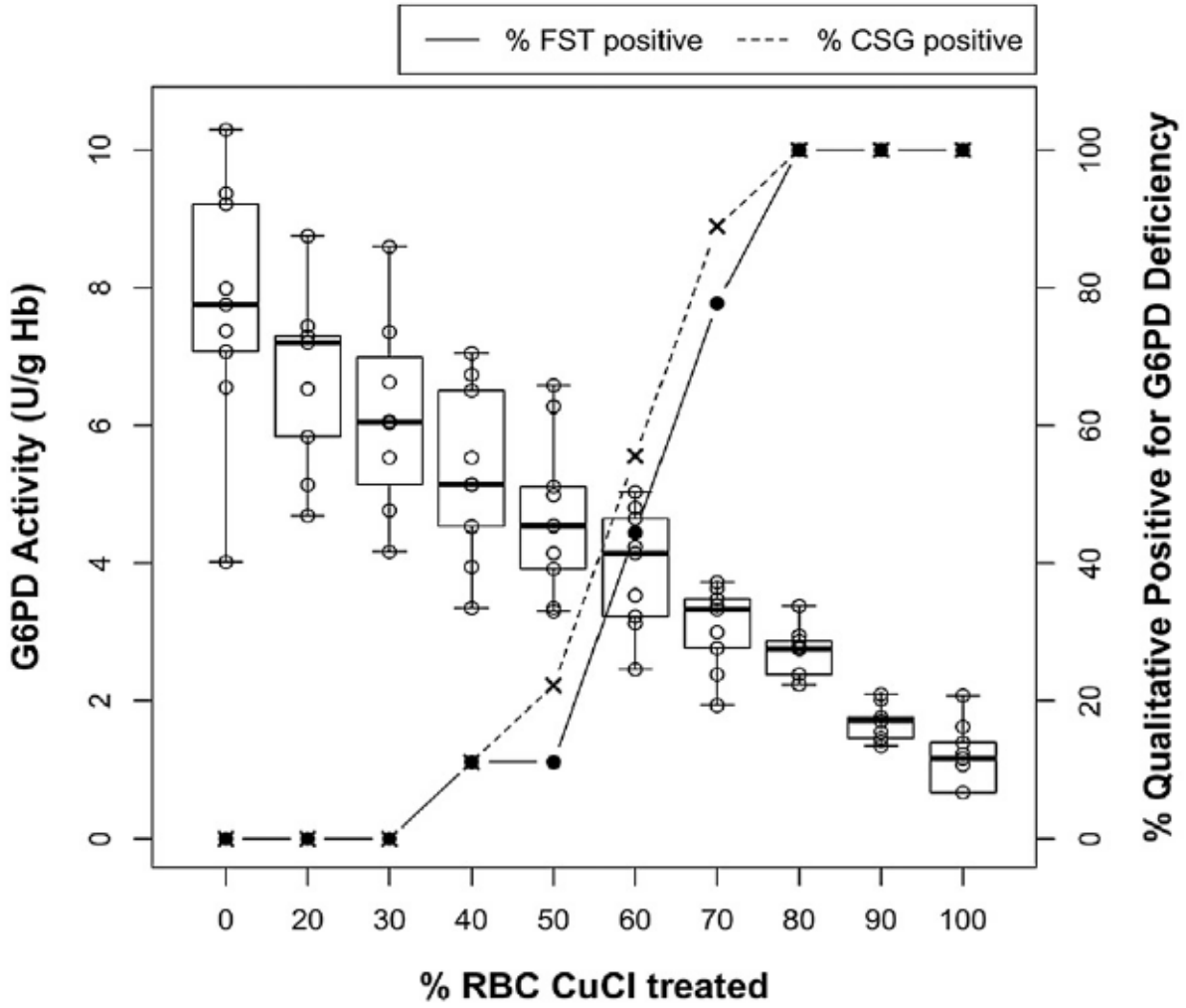


Heterozygous females & G6PD screening



Satyagraha A, et al., PLoS NTD, in press

Heterozygous females & G6PD screening

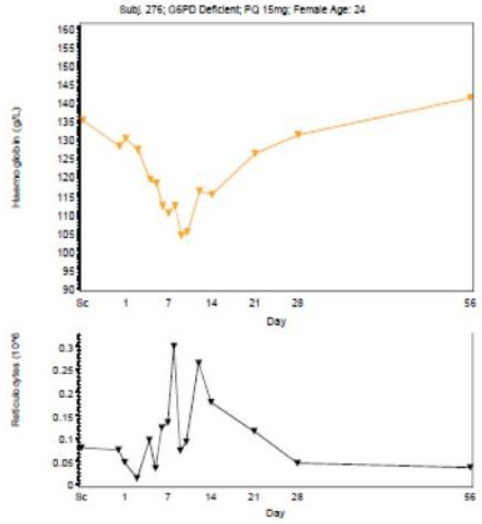


Baird JK et al., Transl Res, 2014

Heterozygous females hemolyze

15%

1



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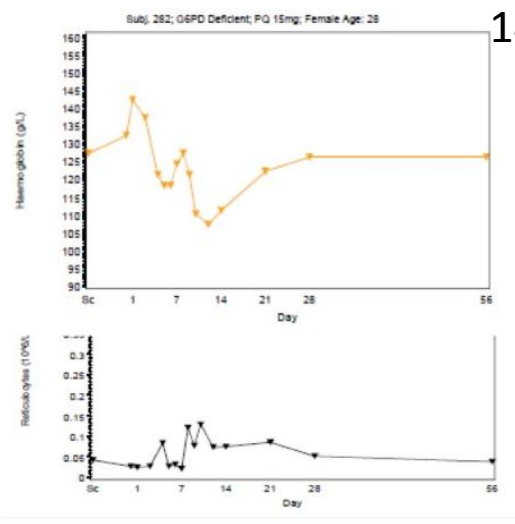
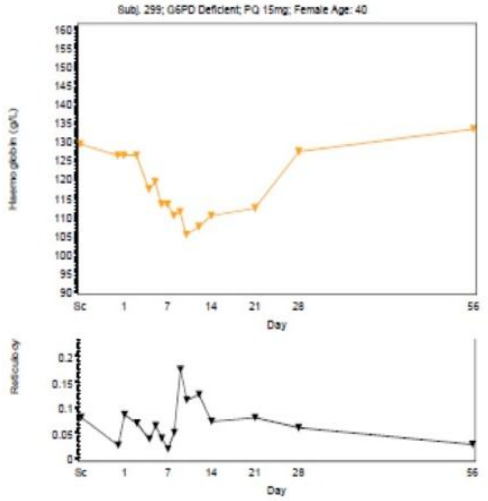


14%

19%

3



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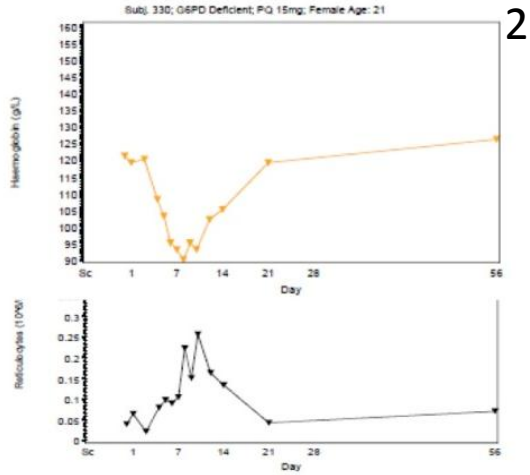


26%

Mahidol variant

40-60% normal G6PD activity

Published evidence POC Dx

Ref	test	specimen	Gold Std	Threshold	# samples	# deficients	heterozygotes	Sensitivity %
1	WST8/1-methoxy PMS	Finger prick /DBS	R&D diagnostics	<60% median of males and females	235	30 (all > 10% normal)		72
2	FST	venous	Trinity	Median of normal males	214	23	25	100 (30%) 91 (60%)
3	FST	venous	BIOLABO SA/	10% genotype	295	42	34	100 (10%) 43 (genotype)
4	*FST*	*?*	*Geno-type*	*All normal by FST*	*461*	*27*	*61*	*All misclassified by FST*
5	Binax NOW	venous	Trinity	4.0 U/gHb	246	50	-	98
6	Binax NOW	venous	Trinity	<60% median of males and females	356	11	-	54.5
2	Binax NOW	venous	Trinity	Median of normal males	214	23	25	100 (30%) 83 (60%)
7	1st gen Care Start	venous	Trinity	Lower limit from 174 normal subjects (~30%	903	97	-	68
8	Care Start	venous	Trinity	Mean from >4.56 IU/gHb and [Hb] >12 g/ Dl	456	46(<30%)	-	90 (<10%) 84.8 (<30%)

Unpublished evidence POC Dx

Study/PI	Test	Sample Type	Setting	Operator	Reader Assessment	Temp (°C)	Sensitivity/ (%)/CI	Specificity/ (%)/CI	PPV/ (%)	NPV/ (%)	Prevalence (%)/ Sample Size	Reference Standard
Cambodia/Menard	CareStart v2	Venous & Capillary	Mobile lab	technician	2 Independent readers, if discordant, a third reader	26–29	100.0	98.7	92.2	100.0	15.0/392	G6PD Quantitative Trinity Biotech
Thailand/Bancone	CareStart v2	Venous	Lab	technician	2 Independent readers, if discordant, a third reader	28–29	87.5	100.0	100.0	89.7	9–18/150	G6PD Quantitative Trinity Biotech
		Capillary					100.0	100.0	100.0	100.0		
Thailand/Bancone	R&D Diagnostic	Venous	Lab	technician		28–29	96.0	100.0	100.0	96.3	9–18/150	
		Capillary					100.0	100.0	100.0	100.0		
Indonesia/Satyagraha	CareStart v2	Venous	Field	technician	1 reader, if unsure, another reader	29–34	100.0/ (100.0–100.0)	98.7/ (97.3–100.0)	89.0/ (77.0–100.0)	100.0/ (100.0–100.0)	9.2/260	G6PD Quantitative Trinity Biotech
Indonesia/Satyagraha	FST Trinity Biotech	Venous in EDTA	Lab	technician	2 readers, if discordant, a third reader	26–29	91.7 / (80.6–100.0)	92.4/ (89.0–95.8)	55.0/ (40.0–70.0)	100.0/ (100.0–100.0)	8.5/260	G6PD Quantitative Trinity Biotech
Brazil/Lacerda	CareStart	Venous in EDTA	Lab	technician	2 readers, if discordant, a third reader	19–26	61.5	98.3	42.1	99.2	1.9/674	G6PD Quantitative Pointe Scientific

Sensitivity = classified as deficient/true deficients

NPV = true deficients/classified as deficients

30% of normal activity set cut-off

Unpublished evidence POC Dx

Study/PI	Test	Sample Type	Setting	Operator	Reader Assessment	Temp (°C)	Sensitivity (%)/CI	Specificity (%)/CI	PPV (%)	NPV (%)	Prevalence/ Sample Size	Reference Standard
Cambodia/Menard	CareStart v2	Venous & Capillary	Mobile lab	technician	2 Independent readers, if discordant, a third reader	26–29	100.0	94.5	36.6	100.0	3.6/419	G6PD Quantitative Trinity Biotech
Thailand/Bancone	CareStart v2	Venous	Lab	technician	2 Independent readers, if discordant, a third reader	28–29	90.9	97.4	90.0	97.4	-	G6PD Quantitative Trinity Biotech
		Capillary					100.0	82.7	60.6	100.0		
Thailand/Bancone	R&D Diagnostic	Venous	Lab	technician		28–29	95.5	97.4	91.3	98.7	-	
		Capillary					100.0	97.4	91.7	100.0		
Indonesia/Satyagraha	CareStart v2	Venous	Field	technician	1 reader, if unsure, another reader	29–34	83.3/ (53.5–100.0)	92.7/ (90.0–95.5)	17.0/ (3.0–30.0)	100.0/ (99.0–100.0)	1.4/350	G6PD Quantitative Trinity Biotech
Indonesia/Satyagraha	FST Trinity Biotech	Venous in EDTA	Lab	technician	2 readers, if discordant, a third reader	26–29	100.0/ (100.0–100.0)	92.2/ (89.3–95.0)	18.0/ (5.0–31.0)	100.0/ (100.0–100.0)	1.7/350	G6PD Quantitative Trinity Biotech

Sensitivity = classified as deficient/true deficients

NPV = true deficients/classified as deficients

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.

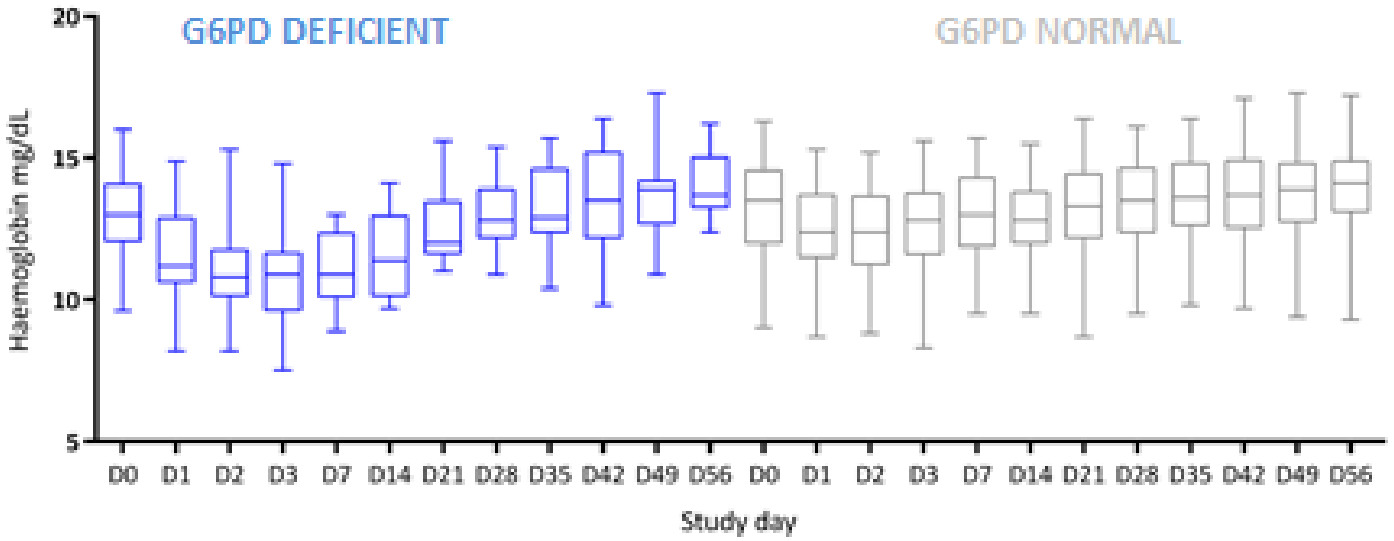
POC tests do not reliably exclude female heterozygotes from risk of AHA with daily primaquine therapy

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

ERG Recommendation #5



* Post transfusion Hbs have been excluded from one G6PDd patient transfused on D5

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