Malaria Policy Advisory Committee (MPAC) Meeting

17-19 October 2017
Chateau de Penthes, 18 Chemin de l'Impératrice, Pregny-Chambésy, Geneva, Switzerland

PROVISIONAL PROGRAMME*

Tuesday, 17 October 2017			
	Session 1	**Open**	**for information**
09:00 – 09:15	Welcome by the Chair, MPAC	Dr Kevin Marsh	
09:15 – 10:30	Report from the Director, GMP	Dr Pedro Alonso	
10:30 – 11:00	*Coffee break*		
	Session 2	**Open**	**For decision**
11:00 – 12:00	Outcomes from ERG on low density malaria infections /Presentation	Dr Andrea Bosman	
12:00 – 12:30	Outcomes from ERG on deployment of PBO plus pyrethroids nets /Information note	Dr Maureen Coetzee	**For information**
12:30 – 14:00	*Lunch*		
	Session 3	**Open**	**for information**
14:00 – 14:30	Update on Malaria Elimination in the GMS	Dr Rabi Abeyasinghe	
14:30 – 15:30	Outcomes from the Drug Efficacy & Response TEG /Presentation	Dr Pascal Ringwald	
15:30 – 16:00	*Coffee break*		
	Session 4	**Open**	**for information**
16:00 – 17:00	Response plan to *pfhrp2* gene deletions/Information note	Dr Jane Cunningham	
17:00 – 17:30	Update on RTS,S pilot implementation/Presentation	Dr Mary Hamel	
17:30 – 18:00	Malaria Vaccine Advisory Committee (MALVAC) update/ Presentation	Dr David Schellenberg	**For decision**
18:00	*End of day*		
Wednesday, 18 October 2017			
	Session 5	**Open**	**for information**
09:00 – 10:00	Update on the Vector Control Advisory Group	Dr Tom Scott	
10:00 – 11:00	Outcomes of the ERG on comparative effectiveness of vector control tools	Dr Azra Ghani	**for decision**
11:00 – 11:30	*Coffee break*		
	Session 6	**Open**	

Report from the Global Malaria Programme

Malaria Policy Advisory Committee

Geneva, Switzerland

Dr Pedro L. Alonso, Director

17 October 2017

Global **Malaria** Programme

World Health Organization

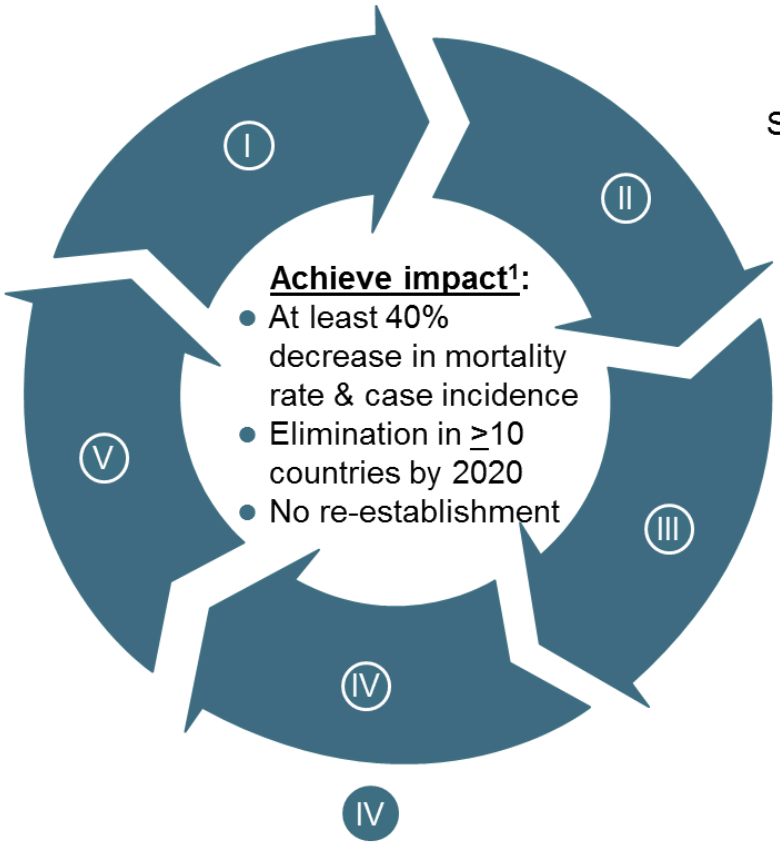
GMP Strategy - Core Roles

I

In collaboration with the malaria community, address key **strategic questions** related to malaria control and elimination

II

Set, communicate & disseminate evidence-based **normative guidance**, policy advice and implementation guidance to support country action



V

Keep an **independent score** of global progress in malaria control and elimination, including **drug & insecticide resistance**

III

Coordinate WHO **capacity building & technical support** to member states, jointly with Regions, ISTs and countries

IV

Help countries develop & implement robust **surveillance** systems to generate quality data and use that data to achieve greater impact

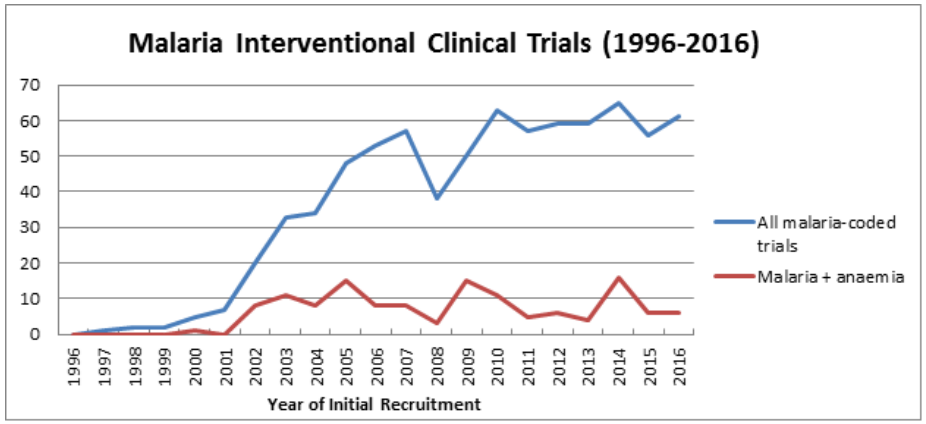
I. Strategic questions

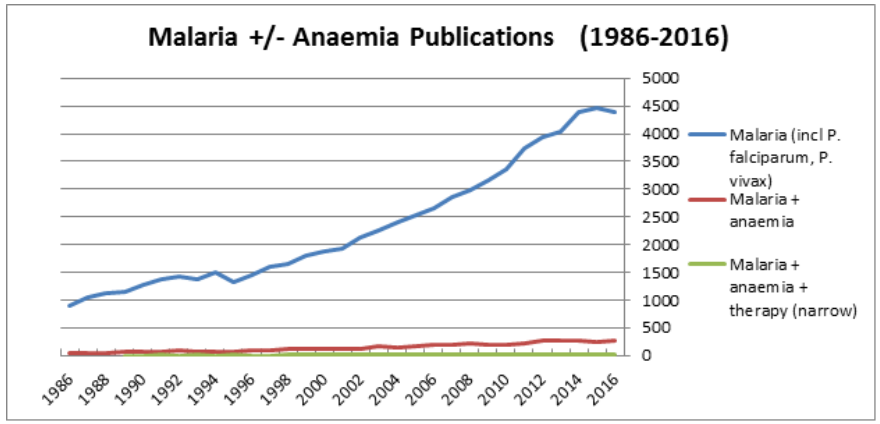
- Global Vector Control Response 2017 - 2030
  - Unanimously welcomed by 70th WHA in May 2017
  - Available in all UN languages

- Strategic Advisory Group on malaria Eradication
  - paper discussed by Executive Board
  - Clarifies terminology and affirmed the Organization's long-standing commitment to eradication
  - Next SAG meeting Nov 30 – Dec 1

I. Strategic questions

- Analysis on malaria mortality
  - Where, why and how to respond
- WHO Global Observatory on Health R&D
- Anaemia and malaria





II. Normative guidance (since last report)

II. Normative guidance (since last report)

The evaluation process for vector control products

JUNE 2017 INFORMATION NOTE

The WHO process for the evaluation of vector control products has been revised to better meet the needs of countries endemic for, or at risk of, vector-borne diseases. The revised process came into effect on 1 January 2017 and is designed to accelerate product evaluation to support the continued scale up of core malaria vector control interventions, to strengthen vector control for neglected tropical diseases, and to address key challenges, such as emerging vector resistance to insecticides.

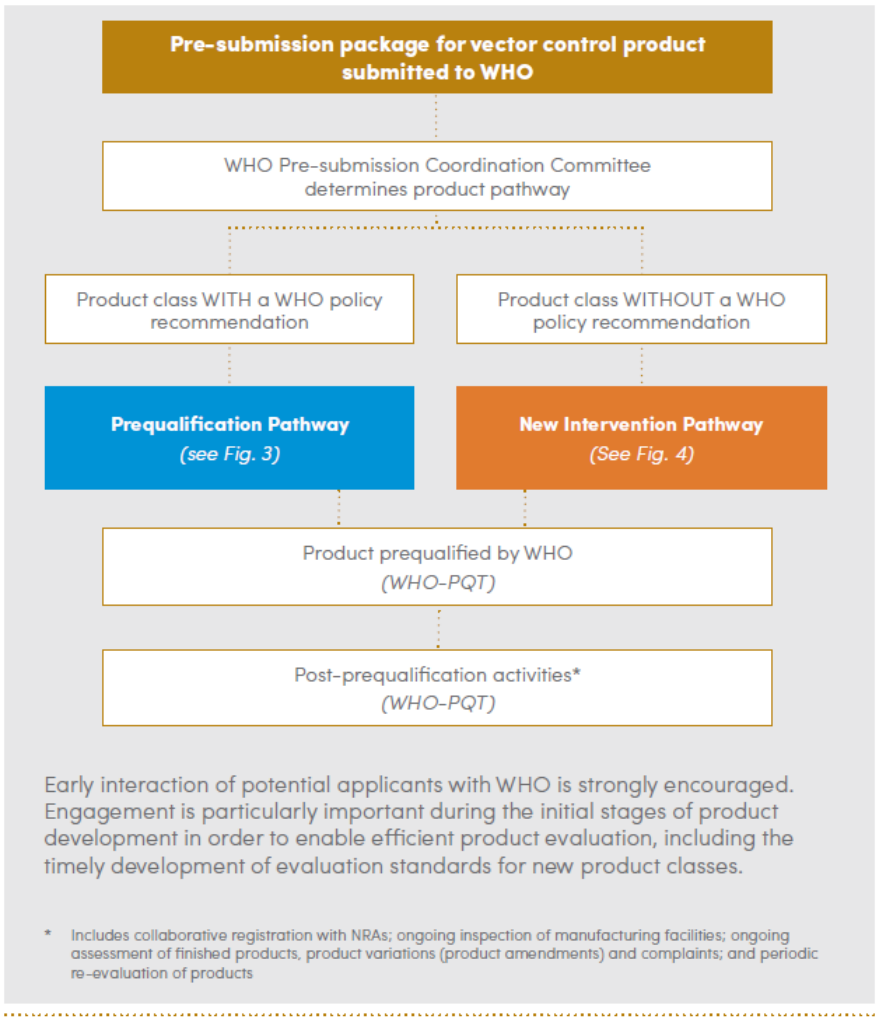
The key objectives of the revised process are to:

1. Enable access to safe, effective and high-quality vector control products;
2. Enhance evidence-based guidance to promote best use and management of vector control tools, technologies and approaches;
3. Promote product quality throughout the product's life cycle.

Under the revised process, the evaluation pathway to be followed is determined by whether or not a product is part of a class with an existing WHO policy recommendation. A policy recommendation is a position statement or recommendation issued by WHO, the most recent of which takes precedence over any previously issued recommendation.

Products covered by a policy recommendation will follow the *Prequalification Pathway*, while all others will follow the *New Intervention Pathway* to validate whether the product has public health value. In the latter case, WHO will issue a policy recommendation once the product's public health value has been validated. Both pathways involve the assessment of supporting data and inspections, and are designed to ultimately result in the prequalification of a product. This prequalification is

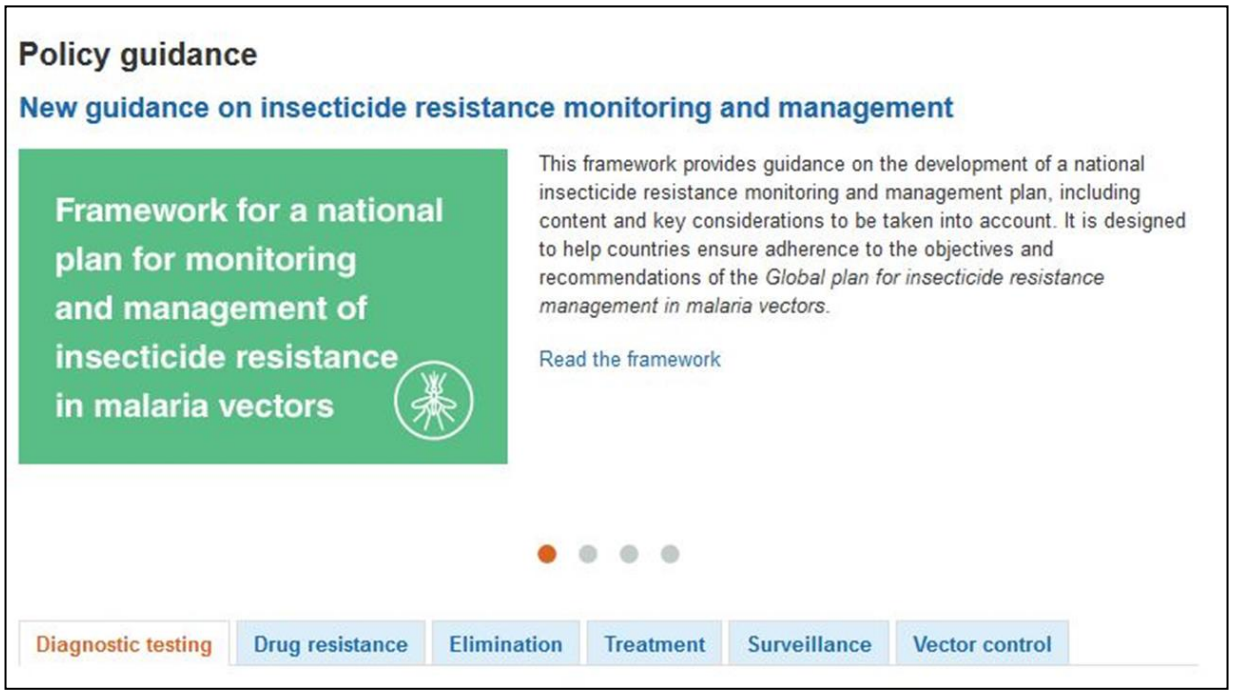
FIG. 1:
Overview of the WHO process for the evaluation of vector control products



II. Normative guidance (since last report)

II. Normative guidance (since last report)

- Strengthening Dissemination
  - New web page showcasing latest guidance
  - Webinars for country staff and partners and available on website; powerpoint available to facilitate discussion



II. Normative guidance (TEGs and ERGs)

Completed in 2017

- Surveillance Monitoring & Evaluation TEG - Feb
- Vector Control TEG - Mar
- Design of epidemiological trials for vector control products - April
- Submicroscopic infections ERG – May
- Vector Control Advisory Group - April
- Drug Efficacy and Response TEG – June
- 2nd ERG on Deployment of PBO + Pyrethroid nets – June
- Malaria in Pregnancy outside of Africa ERG – July
- Comparative Effectiveness of Vector Control Tools ERG – Sept

Planned in 2017 & 2018

- Vector Control Advisory Group - Oct
- Vector Control TEG - Nov
- Chemotherapy TEG – Dec
- Coverage gaps and impact ERG - Dec
- Malaria mortality estimates ERG – 2018
- Malaria MDA in areas of moderate transmission ERG – 2018
- Border malaria ERG – 2018
- Malaria control in complex emergencies - 2018

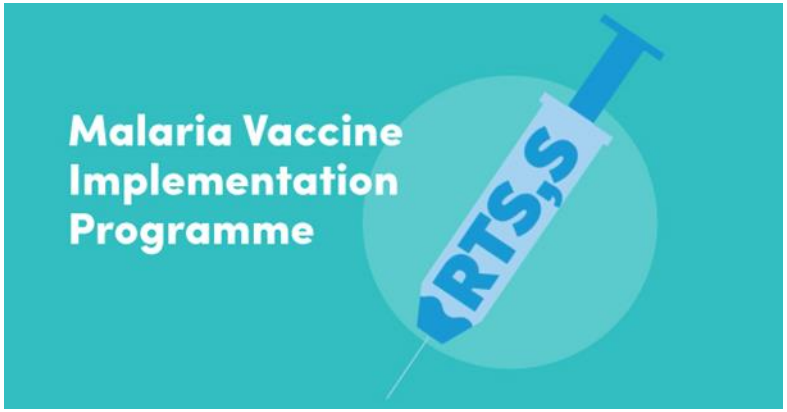
III. Technical Support & Capacity Building

- Technical support provided to countries
  - Surveillance strengthening (41 countries)
  - Elimination and outbreak investigation
  - Updating treatment guidelines
  - Therapeutic efficacy studies
  - Malaria programme reviews and updates to national strategic plans (12 countries)
  - Review and technical input to Global Fund concept notes (50 countries)
  - Emergencies – Nigeria, South Sudan and Yemen

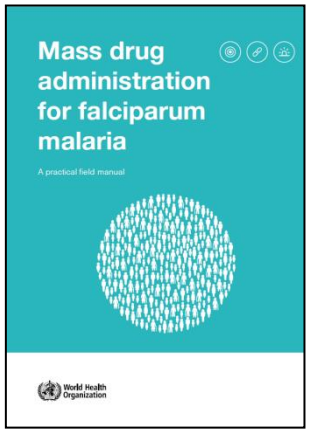
III. Technical Support & Capacity Building (cont.)

Operational support

- Malaria Vaccine Implementation Programme
- Rapid Access Expansion Programme
- Northeast Nigeria
- South Sudan



III. Technical Support & Capacity Building (cont.)



The simplicity in theory of prophylaxis against malaria is only equalled by its difficulty in practice.

Sir Leonard Rogers

Capacity Building

- Strengthening WHO capacity
  - PAHO
  - AFRO (francophone and anglophone)
  - SEARO
- Strengthening country capacity
  - TES training and workshops (25 countries)
  - Surveillance trainings (38 countries in AFRO, EMRO & WPRO)
  - Malaria Elimination (11 countries Asia/Pacific w/JIPD)
  - Entomological surveillance and IVM (EMRO – Nov)
  - African Network on Vector Resistance (16 countries)

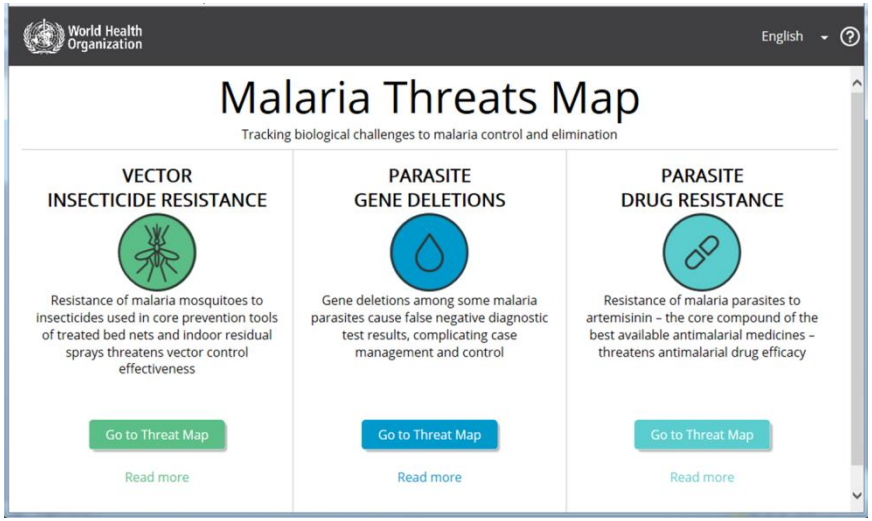
IV. Surveillance

Improving routine data reporting and analysis

- **Surveillance manual –** to be launched Q4 2017
- **Surveillance assessments tools –** to be updated Q4 2017
- **DHIS 2 malaria modules –** completed with data standards for burden reduction and elimination conducted
- **Regional databases –** WPR done, for Q4 2017 (SEAR, AFR, EMR)
- **World Malaria Report** - Substantial systemization of WMR data reporting and analytical approaches

V. Keeping score

- World Malaria Report
  - 29 November launch by DG in New Delhi
  - Supplement on India
- Drug and Insecticide resistance
  - Parasite resistance in GMS – new Q&A online
  - Mapping tool

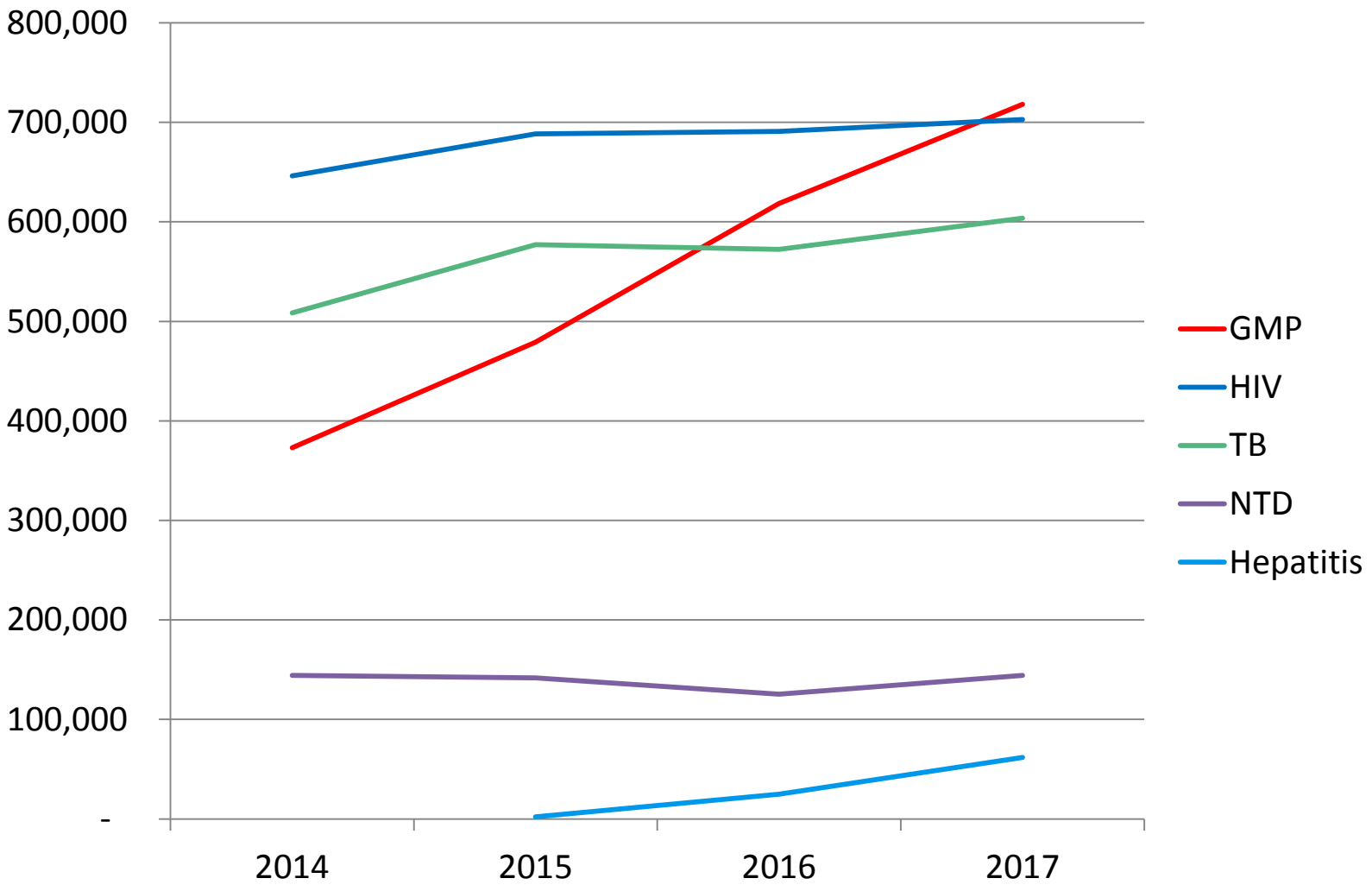


V. Keeping score - GMP website

www.who.int/malaria

- Our most critical information dissemination tool
- Document center provides instant access to key publications
- For the period **1 Jan to 30 Sept 2017**, visits to the GMP site reached **718,123**, up from:
  - **618,346** for the same period in 2016 (**+16%**)
  - **479,011** for the same period in 2015 (**+50%**)

V. Keeping score - Visits to HTM websites



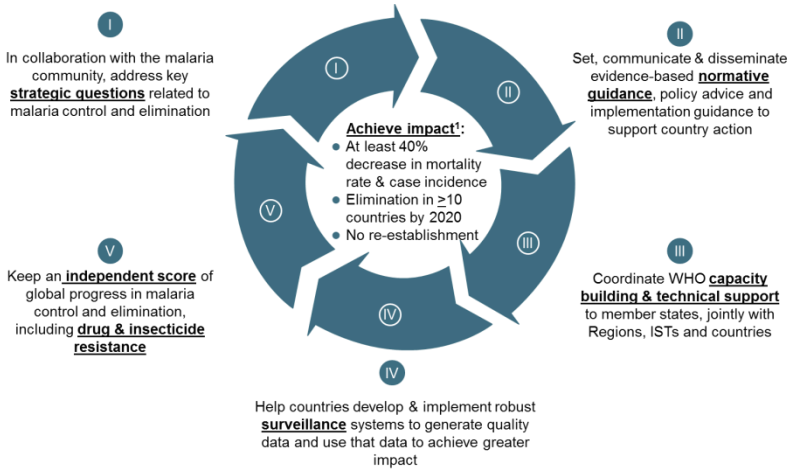
Other GMP updates

- World Malaria Day – high-level forum with Dr Moeti

- Ashgabat Statement – Europe commits to staying malaria free

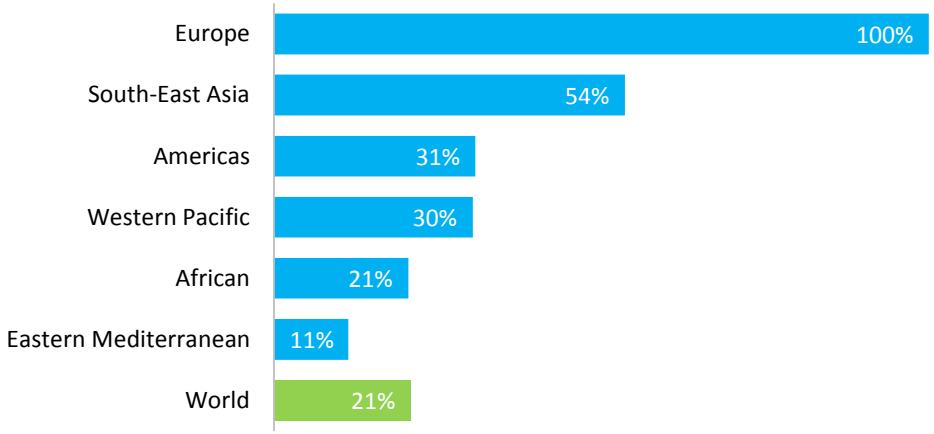
- UNGA side event – leaders commit support for malaria

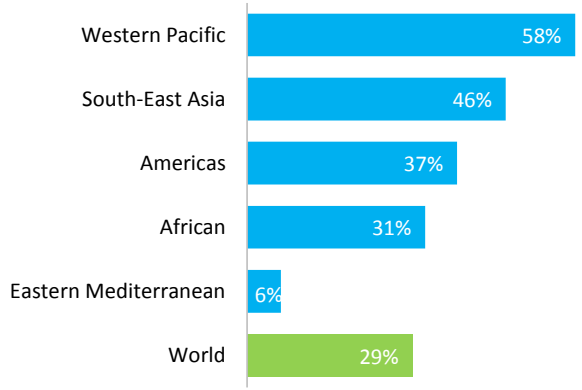
Achieving impact



Achieving Impact by 2020:

- 40% reduction in morbidity and mortality
- Elimination in 10 countries
- Prevention of re establishment





40 of 91 countries on track

39 of 91 countries on track
10 already zero deaths

A problem to be solved not simply a task to be performed

Malaria Policy Advisory Committee Meeting

17–19 October 2017, Geneva, Switzerland
Background document for Session 2

Meeting report of the WHO Evidence Review Group on Low-Density Malaria Infections

15–16 May 2017, Geneva, Switzerland

In March 2014, WHO published recommendations on the use of malaria diagnostics in low transmission settings. Malaria microscopy and antigen-detecting rapid diagnostic tests (RDTs) were recommended as appropriate tools for the diagnosis of clinical malaria and routine malaria surveillance. At that time, WHO recommended that the use of more sensitive nucleic acid amplification (NAA)-based methods should only be considered in epidemiological research and surveys aimed at mapping submicroscopic infections at low transmission intensity and potentially for identifying foci for special interventions in elimination settings. However, WHO also recommended that the use of NAA-based methods should not in any way divert resources away from core malaria prevention and control interventions and the strengthening of health care services, including the surveillance system.

In the years following the publication of these recommendations, the application of NAA-based diagnostic tools in epidemiological research and surveys has expanded and highly sensitive, non-NAA-based point-of-care-methods have been commercialized. Therefore, WHO convened a meeting to revise current recommendations based on a review of the natural history, prevalence, contribution to transmission, and ultimate public health importance of low-density *P. falciparum* and *P. vivax* infections. A report of the meeting proceedings, key conclusions and draft recommendations will be submitted to the WHO Malaria Policy Advisory Committee (MPAC) for consideration.

Conclusions of the ERG:

1. A high proportion of *P. falciparum* and *P. vivax* infections identified in cross-sectional surveys are characterized by low parasite densities undetectable by conventional RDT and microscopy. Although limited by small sample sizes, the relative frequency of low-density infections appears to be higher in low transmission settings than in high transmission ones. The presence of such infections is likely influenced by many factors, including the recent history of transmission, rates of superinfection, genetic diversity of parasites, treatment and immunity. More detailed analyses of existing data and larger datasets from low to very low transmission settings are required in order to improve estimates of the proportion and distribution of low-density infections. Data are limited, and there is great uncertainty regarding estimates in very low transmission settings. More studies are required that also consider the recent history of transmission and potential impact of residual immunity in the population.

WHO/HTM/GMP/MPAC/2017/10

2. Evidence from several reports using mosquito-feeding experiments indicates that mosquitoes can be infected with low-density *P. falciparum* and *P. vivax* infections, although less efficiently than with high-density infections. For *P. vivax*, gametocyte densities closely follow those of asexual parasite stages. Transmission to mosquitoes becomes less efficient at *P. vivax* densities below the limit of detection (LOD) of expert microscopy (estimated at >10 parasites/µl), but can readily occur with infections below the LOD of field microscopy (estimated at >100 parasites/µl). For *P. falciparum*, the relation between gametocyte density transmissibility and the density of asexual parasitaemia is less predictable, and low-density infections below the detection level of expert microscopy can frequently result in mosquito infection. The outcome of experimental mosquito feeds is influenced by a variety of host, vector and parasite factors in addition to methodological factors, but their dynamic interactions are poorly understood.

3. Depending on the relative proportions of low- and high-density infections in a particular location, the role of each in overall transmission may vary considerably. Mosquito feeding experiments help to measure the infectiousness of low- and high-density infections for mosquitoes. However, there are limited data on the relative contributions of low- and high-density *P. falciparum* and *P. vivax* infections to the onward transmission to human populations at the community level. It is critically important to understand the contribution of low-density infections to malaria transmission in order to inform effective malaria control strategies.

4. Conclusive data on the natural history of low-density *P. falciparum* and *P. vivax* infections in different endemic settings remain elusive. Knowledge gaps exist in understanding the longitudinal dynamics of parasite density and infectivity in untreated chronic natural infections; identifying risk factors for carriage of low-density infections; and understanding the prospective clinical and pathological impacts of untreated low-density infections. Available evidence related to the different parasite biology of *P. falciparum* and *P. vivax* suggests that chronicity of infection is achieved through different mechanisms for the two species: antigenic variation and persistence in the blood stream for *P. falciparum*, and periodical relapses for *P. vivax*.

5. With the available evidence, it is difficult to accurately predict how the identification and treatment of low-density *P. falciparum* and *P. vivax* infections through active screen-and-treat based interventions in different endemic settings would impact transmission. Moreover, it is not possible to predict the proportion of the total infectious reservoir that would need to be detected and eliminated in order to accelerate the reduction of transmission. Intervention trials in different epidemiological settings using appropriate control interventions are warranted in order to evaluate the impact on transmission and cost–benefit of applying highly sensitive diagnostics for targeting low-density infections. Until the outcomes of such trials are available, highly sensitive diagnostics should not be part of any routine malaria control or elimination programme; their use should be limited to research purposes.

6. To improve comparability of results, better harmonization and standardization is required in the reporting of the molecular methods used for the detection, identification and quantification of malaria parasites in epidemiological surveys and research studies. Adherence to the Minimum Information for Publication of

Quantitative Real-time PCR Experiments (MIQE) guidelines for reporting quantitative PCR results, as well as the validation of nucleic acid-based amplification assays using standardized and quality controlled material (such as the WHO International Standard for *P. falciparum* DNA NAA Assays) is strongly encouraged. Until standardization is achieved, all reports should include a detailed description of the precise methods used to obtain the data being reported, including the analytical sensitivity and specificity of tests.

7. The terms "submicroscopic," "asymptomatic," and "low-density" infection are often used interchangeably in the literature, generating confusion. "Submicroscopic" generally implies parasitaemia that is below the LOD of microscopy or RDT, but detectable using molecular or other highly sensitive diagnostic methods. The use of the term "submicroscopic" for describing low-density malaria infections should be discouraged. The term "asymptomatic" is not based on parasite density and instead refers to the absence of signs and symptoms of malaria. Asympomatic malaria should be defined with respect to the absence of specific clinical manifestations and the time period evaluated in relation to infection detection. In light of these definitions, the term "low-density" infection is considered most appropriate. When parasitaemia is quantified, a clear definition of "low-density infection" should be reported (suggested: <100 parasites/µl), accompanied by a description of the method of quantification. In studies that do not quantify parasitaemia, low-density infections can be defined as those identified through highly sensitive methods but not detected using conventional diagnostics (microscopy or RDT).

8. Updating the WHO recommendations on the diagnosis of *P. falciparum* and *P. vivax* malaria in low transmission settings is required in order to clarify that WHO does not currently recommend highly sensitive RDTs, other highly sensitive non-NAA-based methods, or NAA-based methods for parasite detection in the routine management of clinical malaria and surveillance. Research is needed to document the public health benefits and cost–effectiveness of detecting and treating low-density infections in low transmission areas and/or specific population groups. In particular, potential research objectives for highly sensitive diagnostics could include epidemiological research to understand the contribution of low-density infections to transmission, border screening of immigrants or migrant populations, foci investigations including the mapping of low-density infections, and use in pregnant women for the detection and treatment of low or sequestered parasite biomass.

   To comply with the above conclusions, the WHO/GMP secretariat in consultation with the ERG Panel Members developed draft recommendations on the diagnosis of *P. falciparum* and *P. vivax* malaria in low transmission settings. These are listed below for consideration by the WHO MPAC.

   8.1. Quality-assured conventional RDT and microscopy are the recommended diagnostic tools for the confirmation and management of malaria cases and malaria surveillance, including routine health information systems and household surveys, in all epidemiological situations. Malaria cases should be reported by type of diagnostic test used.

8.2. A number of highly sensitive techniques are available that detect low-density infections (below 100 parasites/µl). Until there is evidence that the detection of low-density infections using these tools will accelerate malaria elimination, in elimination settings, these tools should only be used for research purposes.

8.3. The majority of infections with asexual parasites have gametocytes detectable by NAA methods, and there is no known benefit of routine detection of low-density gametocytes by molecular methods. All malaria infections (including those infections with low-density parasitaemia) should be considered as potentially infectious.

8.4. Presentation of NAA results should include details of the methods used for sample collection and extraction, and the equivalent quantity of blood added for the PCR reaction, as well as details of outputs in DNA copies or parasite density.

8.5. Before the role of serological assays in malaria elimination programmes can be determined, there is a need for standardization and validation of reagents (antigens and controls), assay methodologies and analytical approaches.

List of abbreviations

CHMI	controlled human malaria infection
FSAT	focal screening and treatment
HRP2	histidine rich protein 2
LM	light microscopy
LOD	limit of detection
MDA	mass drug administration
MIQE	Minimum Information for Publication of Quantitative Real-time PCR Experiments
MSAT	mass screening and treatment
NAA	nucleic acid amplification
PCR, qPCR	polymerase chain reaction, quantitative polymerase chain reaction
POC	point of care
PQ	primaquine
RDT	rapid diagnostic test

1. Background

Quality-assured light microscopy (LM) and rapid diagnostic tests (RDTs) that detect parasite proteins are the basic diagnostic tools currently recommended for the confirmation and management of suspected clinical malaria, as well as for routine surveillance of clinical cases in malaria-endemic settings [1]. After reviewing the evidence in 2013, WHO recommended that the use of more sensitive nucleic acid amplification (NAA) techniques for the detection of low-density malaria infections – i.e., those below the limit of detection (LOD) of LM or RDT – should only be considered for epidemiological research and surveys aimed at mapping low-density infections at low transmission intensity, or for identifying foci to guide intervention measures used specifically in elimination settings [1].

Since then, NAA-based detection of malaria infections has been increasingly applied in surveys and research studies using active or reactive surveillance of populations in endemic areas. The most commonly used method for NAA-based detection in these surveys is amplification of the 18S rRNA gene from finger-prick blood samples [2]. In recent years, quantitative NAA-based methods have often been utilized to quantify parasitaemia in low-density infections below the LOD of LM. For *P. falciparum*, and recently also for *P. vivax*, systematic reviews have concluded that LM misses approximately 50% of infections compared to polymerase chain reaction (PCR)-based detection of parasitaemia [3,4], although this proportion (and the absolute number of missed infections) varies considerably in different epidemiological settings. In cross-sectional surveys, the proportion of low-density infections among all detected infections is higher in low transmission areas than in high transmission areas for both species [4,5]. For *P. falciparum*, it is estimated that, in low to moderate transmission settings, low-density infections account for 20–50% of transmission to mosquitoes [5]; however, a comparable estimate for *P. vivax* is lacking.

Since the publication of WHO's recommendation on the use of malaria diagnostics in low transmission settings, there has been an increasing number of epidemiological surveys evaluating different diagnostic tools for reducing transmission through intervention strategies such as mass screening and treatment (MSAT) or focal screening and treatment (FSAT). In 2015, a WHO Evidence Review Group on mass drug administration (MDA), MSAT and FSAT concluded that current point-of-care (POC) diagnostic tests, MSAT and FSAT are not suitable interventions for interrupting malaria transmission. Funding agencies, manufacturers and researchers have been working towards developing highly sensitive RDTs with LODs similar to those of NAA-based methods. One highly sensitive RDT is now commercially available (Alere™ Malaria Ag P.f RDT, http://www.alere.com), and the manufacturer claims 10-fold higher sensitivity than conventional RDTs and easy deployment at the POC [6].

As more and more countries reduce the burden of malaria and move towards elimination, new evidence on the relevance of low-density *P. falciparum* and *P. vivax* infections in maintaining malaria transmission needs to be reviewed. Additionally, national malaria control programmes require clear guidance on the case management and reporting of low-density infections identified during surveys or as part of research studies. A research agenda is needed to better understand and predict the public health importance of low-density malaria infections and the potential impact of detecting them using highly sensitive RDTs.

2. Objectives

The specific objectives of the meeting were:

1. To review data on the natural history of low-density *P. falciparum* and *P. vivax* infections in different epidemiological settings; to evaluate implications for detectability, duration of infection, and infectivity; and to assess the relationship with symptoms of clinical malaria.

2. To describe the contribution of low-density *P. falciparum* and *P. vivax* infections to transmission at the population level, considering different levels of vectorial capacity and immunity in the population.

3. To define procedures for the case management and reporting of low-density *P. falciparum* and *P. vivax* infections identified through multiple means, e.g., reactive case detection, surveys, research, etc.

4. To review and update the WHO recommendations on the diagnosis of *P. falciparum* and *P. vivax* malaria in low transmission settings; these recommendations were endorsed by the Malaria Policy Advisory Committee in March 2014, based on the report of the 2013 ERG meeting.

5. To establish a set of research priorities and study design characteristics with which to address knowledge gaps on the relative importance of low-density infections and the public health impact of detecting them using highly sensitive diagnostic tests.

3. Process

The Global Malaria Programme / Prevention, Diagnosis and Treatment unit collaborated with Dr. Teun Bousema, Radboud University Medical Center of The Netherlands, and Prof. Chris Drakeley, London School of Tropical Medicine and Hygiene, in the planning of the ERG meeting and selection of studies to meet the specific objectives listed above. WHO commissioned three systematic reviews of the available evidence on the detectability and infectivity of low-density *P. falciparum* and *P. vivax* infections and on the clinical consequences of low-density infections. These pre-reads, together with relevant WHO reports, one unpublished study, and additional relevant published literature were shared with all participants as pre-reads prior to the meeting (Annex 2).

The reviews and background papers were presented and discussed in plenary at the meeting. This was followed by plenary discussions in thematic panel sessions on:

1. The natural history of low-density *P. falciparum* and *P. vivax* infections

2. Contribution to transmission of low-density *P. falciparum* and *P. vivax* infections

3. Clinical management and surveillance of low-density *P. falciparum* and *P. vivax* infections

The first part of the meeting concluded with presentations and discussions on potential programmatic applications of a highly sensitive diagnostic POC test, and on the highly sensitive Alere™ Malaria Ag P.f RDT by the test developers and their partners.

ERG participants were split into three working groups to address specific questions related to 1) natural history, 2) transmission, and 3) the clinical management and reporting of low-density malaria infections. The goals were to establish a set of research priorities, to review and update the current WHO recommendation on the diagnosis of *P. falciparum* and *P. vivax* malaria in low transmission settings, and to propose terms and definitions of low-density malaria infections. Rapporteurs of the working groups presented each group's findings to the whole group for further discussion and consensus-building.

The meeting report was compiled by Dr. Natalie Hofmann, based on the meeting pre-reads and the presentations and discussions held during the ERG meeting. All participants were invited to review the report and provide further input for consideration in finalizing the report.

In terms of objective 4 of the meeting, the outcomes of the working groups were considered separately by the WHO/GMP secretariat in consultation with the ERG Panel Members. A set of draft recommendations on the diagnosis of *P. falciparum* and *P. vivax* malaria in low transmission settings was elaborated for consideration by the WHO MPAC.

4. Evidence review

4.1. The natural history of low-density *P. falciparum* and *P. vivax* infections

4.1.1. Terminology and definitions of low-density malaria infections

The participants of the ERG noted the need for a clear distinction between the terms "submicroscopic," "low-density" and "asymptomatic" infection, as these terms are often used interchangeably both in the literature and in practice. "Submicroscopic" generally implies parasitaemia below the LOD of microscopy, which for *P. falciparum* is comparable to parasitaemia below the LOD of RDT. The term "asymptomatic" refers to the absence of signs and symptoms of malaria and is not based on parasite density. When used, the term should be defined with respect to specific clinical manifestations and the time period evaluated in relation to infection detection. The ERG agreed to promote the use of the term "low-density" to describe infections with low parasitaemia that can occur with and without signs and symptoms of malaria and that may or may not be detectable by LM.

For the purpose of this document, the committee chose to use <100 parasites/µl as a working definition of low-density infection, as this threshold focuses on parasitaemia that lies below the limit of detection of conventional microscopy. The committee, however, acknowledged that expert microscopists can detect parasitaemia below 100 parasites/µL; moreover, in many settings, "routine microscopy" does not necessarily achieve this level of sensitivity.

A clear description of the method applied to quantify parasitaemia and a definition of low-density infection (for example in relation to microscopy or a specific RDT) should be given whenever data are submitted for surveillance or for research publication. For the purpose of this report, the term "low-density" is used to discuss general concepts and future recommendations, while the term "submicroscopic" is used only when referring to specific analyses or publications in which malaria infections were stratified based on their detectability by LM.

4.1.2. Parasite density in P. falciparum *and* P. vivax *infections*

Many studies in recent years have applied molecular methods to quantify parasite density in malaria infections. A great deal of caution should be exercised when comparing parasite densities (and to a lesser extent parasite prevalence) across studies that differ in terms of (i) the method of sample collection and volume of blood sampled, (ii) the duration and conditions of sample storage, (iii) the method of nucleic acid extraction, (iv) the method used for molecular detection, and (v) the copy number of the target sequence. Nonlinearity in the efficiency of nucleic acid extraction and PCR amplification at different parasite concentrations adds further variability and uncertainty to pooled analyses of parasite density estimates. Standard material used to quantify parasitaemia through molecular methods differs among published studies and includes dilutions of target-specific plasmids, field sample DNA, or DNA from synchronized cultured parasites in the case of *P. falciparum*. Particularly for *P. vivax*, where late-stage parasites with multiple genomes are present in the blood stream, conversion between different measures of density is not straightforward (although data from South-East Asia suggest that the presence of mixed parasite stages in the blood does not cause major errors in *P. vivax* density estimates [7]). Although guidelines exist for the reporting of quantitative PCR (qPCR) experiments ("MIQE guidelines" [8]), the reviewed publications often provided insufficient details on the molecular method used for detection.

As more and more studies have applied quantitative NAA techniques for malaria diagnosis in epidemiological surveys, an increasing amount of quantitative parasite density data has become available. In preparation for the meeting, the committee reviewed studies from different endemic settings that used NAA-based methods to quantify parasite density in infected individuals in the community, without selection based on signs and symptoms of malaria or a positive malaria test result. Median *P. falciparum* density by quantitative NAA methods varied between 1 and 1300 parasites/µl. Geometric mean *P. vivax* density in the blood varied between 2 and 50 DNA copies/µl in moderate (Solomon Islands) to high transmission (Papua New Guinea) areas, and between 1 to 213 parasites/µl in low transmission areas of Colombia, Guatemala and Ethiopia.

The density distributions of microscopically detectable and submicroscopic *P. falciparum* infections overlapped in all studies reviewed, highlighting the role of chance and variations in methodology related to both LM and NAA-based techniques. In submicroscopic *P. falciparum* infections, median densities in the reviewed studies ranged from 0.1 parasites/µl to 330 parasites/µl – versus 2 to 9000 parasites/µl in microscopically detectable *P. falciparum* infections. Sufficient data to assess *P. vivax* parasite density in submicroscopic versus LM-detectable infections were only available from Papua New Guinea and Solomon Islands, where the geometric mean *P. vivax* densities ranged between 2 and 8 DNA copies/µl in submicroscopic infections and between 2 and 980 DNA copies/µl in LM-detectable infections.

4.1.3. The proportion of low-density P. falciparum *and* P. vivax *infections in cross-sectional surveys in different endemic settings*

Consistent with previously published findings [3–5,9] and after the inclusion of more recently published studies based on quantitative NAA methods, the two reviews presented at the meeting confirmed that low-density *P. falciparum* and *P. vivax* infections constitute a higher proportion of all infections in low transmission settings than in high transmission

settings. However, given that the absolute number of infections is small in low and very low transmission settings, the absolute number of low-density infections is also smaller than in high transmission settings. In cross-sectional surveys, among the infected population with presence of malaria parasites confirmed by a diagnostic test, the relative proportion of low-density infections was similar for *P. falciparum* and *P. vivax* across the endemicity spectrum (Table 1 shows the proportion of infections that were submicroscopic at different levels of endemicity).

Low-density infections represented at least half of the infections in all transmission settings (>57% of *P. vivax* and >51% of *P. falciparum* infections, Table 1). In addition, for *P. vivax,* a large number of individuals without current blood-stage parasitaemia were infected with hypnozoites that could not be detected. Estimates from Papua New Guinea suggest that approximately 80% of new *P. vivax* blood-stage infections originate from relapsing hypnozoites in tropical areas [10]. These estimates support the presence of a large hypnozoite reservoir in the population.

In the few areas that have monitored low-density infections over time during a period of reduction in transmission, the relative proportion of low-density *P. falciparum* and *P. vivax* infections has increased slightly over time (indicated by an increase in submicroscopic infections) [5–9 and Robinson, unpublished].

TABLE 1.
The proportion of *P. falciparum* and *P. vivax* infections that are submicroscopic at different levels of transmission. Transmission intensity is classified by malaria prevalence assessed using NAA-based techniques.
Data taken from published and unpublished studies assessing *P. falciparum* and *P. vivax* parasitaemia using NAA-based methods (Slater & Okell, Robinson, meeting pre-reads).

	Low transmission 0–10%	Moderate transmission 10–20%	High transmission >20%
P. falciparum			
Number of studies	n = 9	n = 1	n = 8
Unweighted Mean[1] (IQR)	75.0% (77.3–90.4)	not applicable	56.7% (51.4–63.6)
Weighted Mean[2] (CI_{95})	85.4% (81.5–88.7)	72.2 (67.4–76.6)	51.1% (48.7–53.5)
P. vivax			
Number of studies	n = 29	n = 20	n = 15
Unweighted Mean[1] (IQR)	82.5% (68.0–100)	72.6% (59.2–90.7)	57.2% (50.0–73.8)
Weighted Mean[2] (CI_{95})	70.7% (67.5–73.8)	72.0% (70.2–73.7)	58.1% (56.3–59.8)

[1] The unweighted mean is calculated by taking the raw average across all studies, by transmission level, of the proportion of submicropscopic infections observed in each study (independent of study size). The interquartile range is given as a measure of variability in the proportion of submicropscopic infections between studies. For *P. falciparum* only, one study was characterized as "moderate transmission" and the unweighted mean is thus not applicable.

[2] The weighted mean is calculated as an overall proportion of submicroscopic infections from accumulated data by transmission level and reported with a binomial 95% confidence interval.

The majority of low transmission settings are characterized by a high proportion of low-density infections, including areas with historically low transmission, such as Brazil [15,16], Haiti [17] and the Pacific islands [18]. However, a small number of settings with very low transmission (PCR prevalence below 1%) in Haiti [19], China [20], the Brazilian Amazon (Mueller, unpublished) and Solomon Islands [21] are exceptions to this general trend, as most *P. falciparum* infections were detectable by LM. It remains unknown whether after a prolonged period of sustained low-level transmission an inflection point is reached, after which most infections become detectable again by conventional diagnosis, or whether low-density infections that are undetectable by conventional diagnosis will persist. The small numbers of infected individuals per survey in low to very low transmission settings, and the large uncertainty of estimates associated with these small numbers, remain a problem for determining trends at this very low level of transmission. In these very low transmission settings, the choice of population at risk can further influence prevalence estimates, as the few positive cases may be found in small pockets or foci of transmission.

The proportion of infections detected by a diagnostic test further depends on the reference method used. Ultra-sensitive molecular methods, some of which assess high blood volumes [22], have uncovered a larger reservoir of low-density infections than anticipated by standard 18S rRNA qPCR both in a high-endemic area of Tanzania [23] and in low-endemic areas in South-East Asia [24,25]. Along the Thai–Myanmar border, the numerical distribution of parasite densities suggests that, even using ultra-sensitive molecular methods, about 25% of *P. falciparum* infections and 15% of *P. vivax* infections are missed [25].

Based on the parasite density distributions determined using NAA-based methods in the reviewed studies, and recognizing the poor comparability of parasite densities measured using different quantification methods across studies, on average 42% (range 1–97%) and 57% (range 11–100%) of all detectable infections were characterized by parasitaemia above 100 parasites/µl and 10 parasites/µl, respectively (Slater & Okell, meeting pre-read). In the reviewed studies, more than 80% (average of 89%) of *P. falciparum* infections were characterized by parasitaemia above 1 parasite/µl. The proportion of *P. falciparum* infections with low densities below 100 parasites/µl increased in low transmission settings relative to high transmission settings; however, no such trend was observed in the proportion of very-low-density *P. falciparum* infections below 10 parasites/µl.

4.1.4. The detectability of low-density P. falciparum and P. vivax infections in relation to the duration of the infection

Experimental malaria infections are characterized by an initial phase during which rising parasite densities are too low to be detected by conventional diagnostics. This phase is followed in most cases by an LM- or RDT-detectable peak in parasitaemia that often requires treatment. In experimental infections that are left untreated, or where only subcurative doses are applied to mitigate symptoms, the peak in parasitaemia is followed by chronic parasitaemia with fluctuating density, which eventually again falls to low densities undetectable by LM or RDT [26,27].

In data from experimental infections with *P. falciparum* and *P. vivax*, the mean time period between detection of infection by PCR and detection by LM at the start of an infection was approximately 3–5 days in non-immune individuals [28–30]. In a naturally exposed population in Western Kenya, a high transmission setting wherein individuals acquire semi-

immunity with repeated exposure, this period extended to 1 week in young children and to 3 weeks in adults [31].

Long-term persistence of low-level parasitaemia at the tail end of infections is considered more relevant for transmission than the shorter low-density phase at the beginning of the infection. Data from malaria therapy infections indicate a decreasing probability of detection by LM over the course of an infection for both species, but low-density periods (during which the infection is undetectable by LM) can occur early in infection. For *P. vivax*, data from malaria therapy infections have shown decreasing blood-stage densities and infection duration detected by LM with each relapse (i.e., non-primary period of parasitaemia) or homologous reinfection [27].

Data are scarce on the detectability of untreated *P. falciparum* and *P. vivax* infections throughout their duration using LM and/or NAA-based techniques in natural endemic settings. Studying the infection dynamics of natural malaria infections is complicated by superinfection and the interaction of concurrent clones in the host, as well as by host immunity that reduces parasite densities sometimes below the LOD of LM or even PCR [32,33]. For *P. vivax*, relapses contribute substantially to blood-stage infections [10] and add another layer of complexity to parasite detection patterns, even in the absence of superinfection. Using current parasite genotyping methods, it is not possible to distinguish between relapse and primary infection. Novel technologies such as amplicon sequencing [34] provide high-resolution genetic information and have the potential to measure clonal parasite densities, thus overcoming some of the limitations of current molecular methods used to investigate clonal infection dynamics. As such, these novel techniques may help to provide new insights into relapse–reinfection epidemiology and the course of natural infections.

Using statistical methods that take into account periods of low-density parasitaemia below the LOD of PCR, estimates of the mean duration of natural *P. falciparum* infections, per clone, range from several months (e.g., Ghana, 70–200 days [33]; Thailand, 135 days (White, unpublished)) to around 1 month (Papua New Guinea, 36 days (White, unpublished). In studies in Vietnam (Nguyen, unpublished) and Thailand [35], 80–87% of participants remained *P. falciparum*-negative in monthly sampling after one initial detection by ultra-sensitive PCR. By contrast, long persistence of some *P. falciparum* clones over several months was directly observed in Ghanaian infants [36] and over the dry season in Sudan [37,38]. In cohort studies in Africa and Papua New Guinea, low-density *P. falciparum* infections undetectable by LM were preceded and followed by PCR-negative samples in the majority of cases (Slater & Okell, meeting pre-read). In some of these cohorts, but not in others, the presence of such submicroscopic detections was a positive predictor for later LM detections (Slater & Okell, meeting pre-read).

Mathematical modelling suggests that individual *P. vivax* clones persist in the blood stream for 24–29 days in Thailand and Papua New Guinea, with relapses occurring every 41–55 days (White, unpublished). Although each individual blood-stage *P. vivax* infection seems shorter than those of *P. falciparum*, it is conceivable that *P. vivax* achieves comparable persistence through relapse. In Cambodia, untreated infections of *P. vivax* (detected by ultra-sensitive PCR) persisted over several months with densities fluctuating around the LOD of LM [35]. Preventing relapses through treatment with 8-aminoquinolines would effectively shorten *P. vivax* parasitaemia.

It cannot be determined from the majority of available data whether the longitudinally observed patterns in *P. falciparum* and *P. vivax* densities reflect ongoing infections, frequent superinfections due to high exposure, or relapse in the case of *P. vivax*. Across cohorts with data that were available and reviewed, the majority of participants that repeatedly carried submicroscopic *P. falciparum* or *P. vivax* infections became slide-positive at some point during follow-up. Only 0.8–18% of *P. falciparum* and 14–18% of *P. vivax* carriers remained submicroscopic throughout follow-up (Slater & Okell, Robinson, meeting pre-reads).

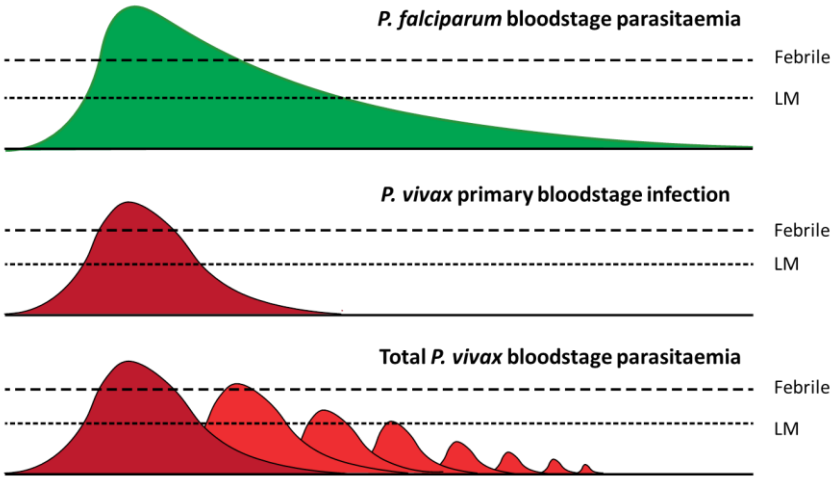


Figure 2. Models of the average pattern of *P. falciparum* and *P. vivax* blood-stage infection dynamics. Blood-stage parasitaemia is depicted in the absence of superinfections. Within individual infections, there are fluctuations in density. Figure taken from the ERG presentation by Ivo Mueller.

4.1.5. The duration of infectiousness in P. falciparum and P. vivax in treated infections

In accordance with their different infection dynamics, schizonticidal antimalarial treatment (i.e., acting only on the asexual blood stages) influences infectiousness in different ways for *P. falciparum* and *P. vivax*. Treatment of a *P. falciparum* infection truncates the infection and interrupts further generation of new gametocytes that contribute to transmission events. Residual circulating mature *P. falciparum* gametocytes maintain transmission in the short-term after treatment, with the duration of gametocyte persistence dependent on the type of (artemisinin combination) therapy used [39]. Primaquine (PQ) treatment reduces the risk of post-treatment transmission of the infection [40]. Treatment of *P. vivax* infections using only blood-stage-clearing drugs does not have an effect against transmission events occurring from subsequent relapses. Only clearance of liver hypnozoites can abrogate future infectivity of *P. vivax* carriers [41,42].

Key conclusions

- The terms “submicroscopic”, “asymptomatic” and “low-density” infection are often used interchangeably in the published literature. Harmonization of terminology and definitions should be promoted. The ERG agreed that “low-density infection” is the preferred term and it should be defined in each publication by stating the applied parasitaemia cut-off (suggested: <100 parasites/µl) along with the molecular method used for quantification. In studies that do not quantify parasitaemia, low-density infections can be defined as those identified through highly sensitive methods but not through conventional diagnostics. Similarly, the use of the term “asymptomatic” should be defined in terms of the specific symptoms recorded and the time period evaluated in relation to infection detection.
- Standardized reporting and harmonization of molecular methods is needed to ensure accuracy of results and comparability between studies. In particular, the LOD of the NAA method (determined using quality-assured reference materials such as the WHO international DNA standard for *P. falciparum*) and the equivalent of blood volume added to the NAA reaction should be specified. A detailed description of the molecular workflow should consist of the specification of the sample type, including the volume sampled, storage conditions, extraction method, amplification method and identifier of target sequence.
- Approximately half of *P. falciparum* and *P. vivax* infections detected in cross-sectional surveys were detected by conventional diagnostics. The proportion of infected individuals with low-density malaria infections detected by more sensitive tests increased in low transmission settings, although absolute numbers of carriers were higher in high transmission settings. There are limited data and high uncertainty with regard to estimates in very low transmission settings. More studies are required that also consider recent history of transmission and the potential impact of residual immunity in the population.
- There are indications that new infections are more likely to be detected by conventional diagnostics than chronic infections, which tend to have lower parasite densities. Infection dynamics of natural infections remain understudied due to limitations of the current molecular genotyping methods and the complexity of required study designs. A better understanding of the longitudinal dynamics and detectability of infections is relevant, particularly in low-endemic settings where superinfections are rare.

4.2 The contribution to transmission of low-density *P. falciparum* and *P. vivax* infections

4.2.1. Factors influencing the likelihood of P. falciparum *and* P. vivax *transmission to mosquitoes*

The wider use of molecular methods to detect and quantify *P. falciparum* and *P. vivax* gametocytes in epidemiological surveys, complemented by experimental mosquito feeding studies, has generated evidence to evaluate the infectiousness to mosquitoes of various parasite and gametocyte densities.

For *P. vivax,* changes in gametocyte densities closely follow those of asexual stages. A variety of studies have shown a clear correlation between *P. vivax* gametocytaemia and total parasitaemia, including in the low-density range, with an asexual parasite to gametocyte ratio of 10:1 [43–45]. The presence and density of *P. falciparum* gametocytes are less well-correlated with asexual parasitaemia because of the long gametocyte maturation and circulation time, and sequestration. Therefore, no clear relationship exists between asexual density and concurrent gametocyte density for *P. falciparum* [44], although a trend of lower *P. falciparum* gametocyte densities has been observed in low-density infections compared to LM-detectable infections (Slater & Okell, meeting pre-read).

Because of their better reproducibility and standardization, as well as due to ethical considerations, membrane feeding assays (MFAs) are commonly used in epidemiological studies rather than direct feeding on skin. However, MFAs do not capture all the elements of mosquitoes' natural skin feeding that might influence transmission. A variety of vector, host and parasite factors further influence the outcome of mosquito feeding experiments. These include (but are not limited to) (i) vector species, density and age; (ii) host immunity, age and symptomatic status; and (iii) parasite and gametocyte density. Few studies have investigated the relevance and individual impact of each of these factors [46], which remain poorly understood and are setting-dependent.

For *P. falciparum* and *P. vivax*, the likelihood of mosquito infection in experimental feeding experiments increases with increases in gametocyte density, exhibiting an S-shaped dose-response relationship; at very low gametocyte density, there is a low likelihood of mosquito infection, whereas above gametocyte densities of 200 to 1000 gametocytes/µl, there is saturation without further increase in the prevalence of infected mosquitoes [43,47–49]. For *P. vivax*, studies in Thailand [49] and Ethiopia (Tadesse, unpublished) described a steep increase in the probability of mosquito infection at a parasite density of approximately 10 parasites/µl. This level coincides with the LOD of expert microscopy. These studies showed that low-density *P. vivax* infections below this threshold were unlikely to transmit to mosquitoes, whereas LM-detectable infections frequently transmitted to mosquitoes, generating high infection rates. As a result, field microscopy with an LOD of around 100 parasites/µl may miss *P. vivax* densities that are readily infectious to mosquitoes. For *P. falciparum, s*uccessful transmission events have frequently been observed at low parasite or low gametocyte densities, but the variation in infectivity data is high [43,47,48].

The available evidence does not facilitate the evaluation of the influence of vector species, parasite strains, epidemiological settings and the host's symptomatic status on the relationship between parasitaemia or gametocyte densities and the likelihood of transmission to mosquitoes. Current experimental systems are limited to investigating human-to-mosquito transmission, but cannot provide information about the likelihood of subsequent mosquito-to-human transmission. Although there are few data on the probability of host infection after exposure to mosquitoes with varying sporozoite loads in the salivary gland, available data indicate a saturating relationship in both *P. berghei* and *P. falciparum* [50]. While the current evidence suggests that even single oocyst infections in *P. falciparum* give rise to hundreds or thousands of sporozoites in the salivary glands [51], and recognizing that oocyst densities in the majority of wild-caught *Anopheles* range between one and three oocysts [52], understanding the likelihood of secondary infections from mosquitoes with a low infection burden is of importance for understanding the relevance of low parasite densities to malaria transmission.

The natural history of infection and longitudinal infection dynamics need to be considered in evaluating the transmission potential of natural infections. While infectiousness was extensively studied in early malaria therapy studies, in all more recent studies, it has been assessed at one point in time without accounting for the dynamic nature of malaria blood-stage infections characterized by oscillating density. Longitudinal studies of the infectivity dynamics of natural infections are lacking.

4.2.2. The P. falciparum *and* P. vivax *low-density infectious reservoir*

To estimate the contribution to transmission of the low-density *P. falciparum* infectious reservoir, i.e., the combined infectivity of a population to mosquitoes [53], data were available for review from five recent studies in high transmission areas in Burkina Faso, Kenya and Senegal, with slide prevalence ranging from 26% to 49% (Goncalves, in press; [54,55]). In these studies, 32–65% of *P. falciparum* infections detected by NAA-based methods were not detectable by LM; these contributed an estimated 15–30% of mosquito infections (Slater & Okell, meeting pre-read). In a re-analysis of data from a study in Kenya with 84% slide prevalence [56], *P. falciparum* infections not detectable by LM were rare and only contributed an estimated 2.3% of mosquito infections. In these six studies the proportion of the infectious reservoir not detected by LM but detected using diagnostics with different LODs increased by 10–30% using a diagnostic with an LOD of 100 parasites/µl and by 70–80% using a highly sensitive diagnostic with an LOD of 1 parasite/µl. Including the LM-detectable infectious reservoir, it is estimated that a highly sensitive diagnostic with an LOD of 1 parasite/µl would detect 83–96% of the total infectious reservoir.

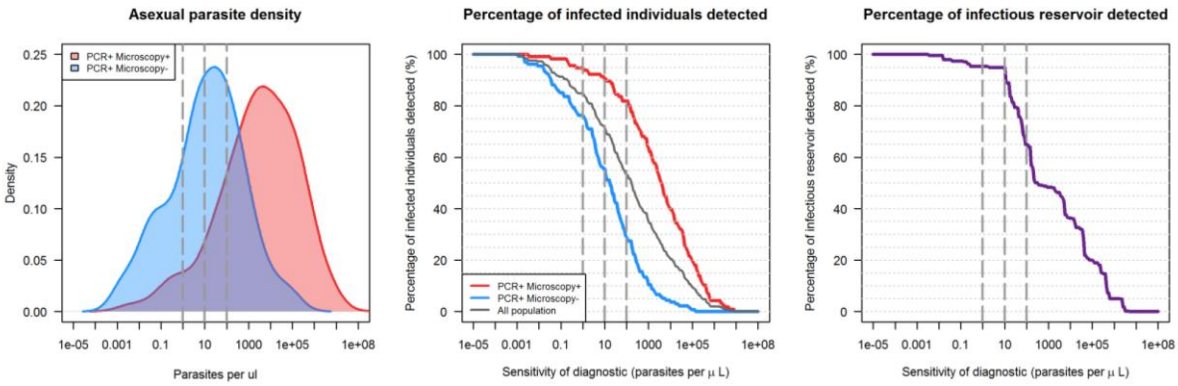


Figure 3. Parasite density distributions, determined using quantitative NAA methods, for individuals from Burkina Faso that are detectable (red) and undetectable (blue) by microscopy (left panel); and the proportion of infected individuals (middle panel) and of the infectious reservoir (right panel) detected at different diagnostic sensitivity thresholds (1, 10 and 100 parasites/µl). Figure from Slater & Okell, meeting pre-read, unpublished data.

The contribution of the low-density infectious reservoir to maintaining malaria transmission is estimated to be higher in areas of low or moderate parasite prevalence than in areas of high parasite prevalence [5], and depends on a variety of vectorial and environmental factors as well as population immunity. Vector species, vectorial capacity and local environmental factors, such as the presence/absence of mosquito–human contact sites, quality of health systems and rate of treatment of infections, can impact the likelihood of onward transmission of low-density infections to mosquitoes. It is currently unclear as to which of these factors are most relevant for maintaining transmission in different endemic

settings, and which are particularly relevant or limiting for the transmission of low-density infections. Most infectivity studies to date have been performed in high transmission settings and comparable studies are lacking in low or moderate transmission settings. In low transmission settings, the screening of large populations is required in order to identify parasite carriers. This is an expensive and labour-intensive task. In addition, quality control for feeding procedures is more complex in low-endemic areas. Quality control of transmission studies requires the recruitment of high-density gametocyte carriers (for which the proportion of infected mosquitoes that can be expected is reasonably well described); however, these high-density gametocyte carriers are less common in low-endemic settings compared to moderate- and high-endemic settings. Infectivity studies may therefore be more feasible in moderate transmission settings than in low transmission settings.

Key conclusions

- For both *P. falciparum* and *P. vivax*, the likelihood and intensity of mosquito infection is positively, but not linearly, associated with gametocyte density. Transmissibility to malaria vectors is less efficient at very low gametocyte densities and plateaus above certain high levels of gametocyte density. The host, parasite and vectorial factors that modify this relationship are not well understood. Critical data are lacking on the likelihood of subsequent mosquito-to-human transmission, and the relationship between sporozoite load and the probability of human infection in natural infections of the human malarias.

- For *P. falciparum*, but less so for *P. vivax*, transmission events occur regularly at low parasite densities below the LOD of expert microscopy (estimated at 10 parasites/µl). At the LOD of non-expert or field microscopy, *P. vivax* parasite densities that are readily infectious to mosquitoes can be missed. There is no evidence of a measurable parasite density threshold below which transmission cannot occur.

- Evidence from a limited number of areas with a high prevalence of *P. falciparum* indicates that low-density infections can be responsible for more than 15% of mosquito infections in these settings. Evidence is lacking from areas with low *P. falciparum* prevalence and for *P. vivax*.

- Current evidence is insufficient for understanding the contribution of low-density *P. falciparum* or *P. vivax* infections to onward transmission to human populations. Intervention trials to directly assess the effect of identifying and treating low-density infections are warranted.

4.3 Relevance of detecting low-density P. falciparum and P. vivax infections

Based on the available evidence, the ERG participants discussed the relevance of detecting low-density *P. falciparum* and *P. vivax* infections in different endemic settings as part of research activities, surveillance or intervention strategies.

It was agreed that the detection of low-density infections has no current role in the case management of suspected malaria clinical cases or in the surveillance of clinical malaria

cases. Currently, there is no evidence to support the use of a highly sensitive HRP2-based POC test for the diagnosis of clinical malaria or surveillance. Conventional RDTs and quality LM are sufficiently sensitive to detect *P. falciparum* densities most commonly associated with the signs and symptoms of clinical malaria.

Considering that in low transmission settings (including those targeted for elimination) low-density infections account for a high proportion of the total number of infections in cross-sectional surveys, and as there is currently no known measurable parasite density threshold below which transmission cannot occur, research is needed to explore the potential impact and cost–effectiveness of highly sensitive POC tests, such as a highly sensitive RDT in active or reactive case detection strategies, for reducing transmission.

Research should target scenarios in which the detection of low-density infections may be most relevant: epidemiological field trials to measure the impact of identifying and treating all infections, including low-density infections, on transmission, border-screening of immigrants or migrant populations, and foci mapping and investigations. Other potential scenarios may include the screening and treatment of pregnant women in antenatal care in order to study how low or sequestered parasites undetectable by conventional diagnostics impact pregnancy outcomes. The potential benefits of detecting and monitoring low-density infections may be specifically investigated in areas where antimalarial resistance occurs, in order to assess the dynamics of natural infections in relation to changes of resistance markers and their role in the development and spread of resistance.

Intervention strategies targeting low-density infections are not applicable in high-transmission settings and carry an increased risk of significant resources being diverted away from clinical case management and conventional diagnostic tests, which are more cost–effective in these settings.

Key conclusions

- Highly sensitive tests have no proven benefit over conventional diagnostics in routine malaria case management and the surveillance of clinical cases.
- Research is needed to document the public health benefits and cost–effectiveness of detecting and treating low-density infections in low transmission areas and/or specific population groups. In particular, potential research objectives for highly sensitive diagnostic tests may include: epidemiological research to measure the impact of identifying and treating all infections, including low-density infections, on transmission, border-screening of immigrants or migrant populations, foci mapping and investigations in the context of malaria elimination, and the detection and treatment of low or sequestered parasite biomass in pregnant women.

4.4 Clinical management and surveillance of low-density *P. falciparum* and *P. vivax* infections

Discussions focused on individual versus community risks and the benefits of treating low-density asymptomatic infections. Ethical considerations with respect to the need for follow-

up or treatment of low-density asymptomatic infections in research settings were also discussed.

4.4.1. Clinical consequences of low-density P. falciparum and P. vivax infections

Persistent asymptomatic malaria infections can contribute to a range of clinical consequences, including (but not limited to) repeated acute illness episodes, all-cause morbidity and mortality (indicated by an excess reduction of morbidity and mortality due to malaria control interventions), malaria-related anaemia, splenomegaly, placental malaria with consequences for both mother and infant, coinfection with invasive bacterial disease, and cognitive impairment [57].

The ERG participants agreed that the current evidence is not sufficient to evaluate the clinical consequences of low-density asymptomatic infections with respect to the natural history of the individual infection. Low-density infections may represent chronic, self-resolving, or pre-recrudescent infections. Currently, it is not known whether a detected low-density infection is a marker of previous or future symptomatic malaria, or a marker of previous exposures to malaria and thus cumulative immunity, and how this relationship changes in different endemic settings.

Asymptomatic low-density infections may be important for maintaining clinical immunity in the presence of ongoing exposure. A recent study in Mali found that, for children initially carrying a chronic asymptomatic *P. falciparum* infection, the risk of clinical malaria was reduced over two transmission seasons compared to children without a diagnosed malaria infection. This reduction in risk was comparable for children in whom the chronic infection was treated (RDT-positive children) and for children in whom infection was allowed to persist (RDT-negative, PCR-positive) [58]. In one recent study in Zambia, asymptomatic and symptomatic malaria infections appeared to be associated with genetically distinct parasite subpopulations [59]. More studies are required to evaluate the relevance of chronic low-density malaria infections for maintaining clinical immunity in different endemic settings.

4.4.2. Treatment of low-density P. falciparum and P. vivax infections in research versus programmatic settings

There is a large body of evidence supporting the negative clinical consequences of asymptomatic *P. falciparum* and *P. vivax* infections [57], as well as the role of submicroscopic malaria infections in defining the human infectious reservoir for *P. falciparum* malaria [5]. There is, however, limited evidence on the prospective clinical and pathological impact of asymptomatic low-density infections that are undetectable by conventional diagnostics. In programmatic settings, the risks and benefits of treating asymptomatic, low-density infections have to be weighed at both the individual and the community level.

At the individual level, every malaria infection detected, irrespective of parasite density, should receive appropriate treatment to prevent future morbidity and mortality. The ERG agreed that at the individual level this benefit outweighs the risks associated with treating the infection; such risks may be related to drug adverse effects or the loss of the potential protective effect of chronic infection against clinical malaria. However, given the current state of knowledge, the added cost of seeking, finding and treating low-density malaria infections (detected in asymptomatic individuals or patients presenting with fever of non-malarial origin) should not divert resources away from the management of symptomatic

malaria cases and other components of national malaria control programmes. Studies to determine the impact and cost–effectiveness of treating low-density infections in routine clinical practice or surveillance are essential for guiding the use (and subsequent treatment actions) of highly sensitive POC diagnostics in high to medium transmission settings.

In research settings, case management and treatment of low-density malaria infections should generally be provided according to the research protocol approved by the national ethics review committee. In research settings, infections are frequently not detected at the POC but retrospectively, making it operationally challenging to trace the participants and treatment. Where the aim of the research activity consists of monitoring longitudinal aspects of infections, provision of antimalarial treatment at enrolment or during follow-up may interfere directly with the research aim. Given that there is limited evidence to indicate that low-density infections are associated with significant future malaria morbidity, treatment of asymptomatic infections identified at study contact in research settings may be withheld after consultation with national ethics review committees, provided that positive participants' signs and symptoms of malaria can be closely monitored. Appropriate care should be given to infected individuals presenting with symptoms. If infections are identified retrospectively, every effort should be made to raise awareness in the study area on the risks and symptoms of malaria infection and encourage appropriate care-seeking behaviour.

4.4.3. Reporting of low-density P. falciparum *and* P. vivax *infections in the surveillance system*

The availability and use of a highly sensitive RDT as part of active or reactive case detection in malaria programmes is likely to result in the increased detection and reporting of low-density parasitaemias. The ERG agreed that malaria cases found through active or reactive case detection should be reported separately from those detected passively, preferably along with the mode of diagnosis and the denominator of the population screened. When reporting, the diagnostic method used should be indicated (e.g., conventional RDTs, LM, highly sensitive RDTs or specific NAA-based methods). In addition, the type of diagnostic used should be taken into account in trend analysis, intervention targeting and impact evaluation. The comparability of measures between years is crucial for trend analysis.

Further research is required to identify the most cost–effective deployment strategy of highly sensitive diagnostics for malaria surveillance. A better understanding of the proportion of low-density infections that need to be identified and treated in order to reduce transmission in different transmission and epidemiological settings is crucial for designing cost–effective implementation strategies. Controlled trials of active and reactive case detection (such as FSAT or MSAT using highly sensitive diagnostics) compared to relevant interventions (such as MDA, reactive case detection using conventional diagnostics, or universal access to diagnosis and treatment and vector control) are required to assess the potential role of highly sensitive diagnostics in accelerating malaria elimination. These trials will generate the evidence to inform future WHO recommendations on detection schemes of low-density infections for malaria elimination and certification of malaria-free status.

Key conclusions

- A significant proportion of asymptomatic infections are characterized by parasite density that is below the LOD of LM or conventional RDT. Low-density infections are frequently detected also in febrile patients, particularly in high-endemic areas, but these may not be the underlying cause of fever. The available evidence is not sufficient to fully evaluate the prospective clinical and pathological impact of untreated low-density malaria infections.
- In programmatic settings, every detected malaria case (including low-density malaria infections) should receive appropriate treatment. Appropriate treatment should include PQ for P. vivax cases.
- In research settings, appropriate care should be given to infected individuals in line with national ethics committee requirements. In research scenarios in which low-density infections are identified retrospectively or in which treatment would directly interfere with the study aim, treatment of asymptomatic malaria cases may occasionally be withheld only if close monitoring for signs and symptoms of acute malaria is provided.
- Malaria cases identified by active or reactive case detection should be reported separately from those detected passively, along with the mode of diagnosis and the denominator of the population screened.
- Controlled trials of active and reactive case detection using RDTs and highly sensitive RDTs are required in low transmission settings in order to assess the impact on transmission of detecting and treating low-density asymptomatic infections, and to design cost–effective strategies for their use by malaria programmes.

4.5. Priority research questions

There is a need to better understand the contribution of low-density infections to transmission to human populations in endemic communities, and to directly evaluate the impact on transmission by actively detecting and treating low-density infections in intervention trials in different endemic and epidemiological settings.

Specific research questions:

- What is the proportion and absolute number of low-density infections in low and very low transmission settings (0–5% prevalence by PCR), and what is the spatial distribution of malaria infections?
- What is the relationship between the proportion of low-density infections and recent history of transmission, i.e., is an inflection point reached in the proportion of low-density infections detected by highly sensitive diagnostics in areas with sustained reduction of transmission at very low levels?
- What is the proportion of low-density asymptomatic infections that become symptomatic as part of the natural history of infection in different endemic settings?

- What is the prospective clinical and pathological impact of untreated low-density parasitaemia?
- What are the risk factors for persistence, and what is the role of low-density infections in the spread of antimalarial resistance?
- Can novel molecular techniques such as amplicon sequencing aid in investigating the natural history of infections, e.g., by measuring clonal parasite density, and in investigating relapse–reinfection epidemiology?
- In the natural history of infections, what is the duration of infectiousness (particularly in low-endemic settings) and what are its major determinants?
- What are the main determinants – related to host, vector and parasite – of infection success in experimental mosquito-feeding experiments and of making those mosquitoes infectious for humans? What is the relationship between parasite density and infectiousness for different vector species? What are feasible study designs with which to achieve meaningful numbers in low-endemic settings?

The participants agreed that many of the research questions listed above are unlikely to be answered within the timeframe required to form an evidence base for guiding malaria control programmes and elimination strategies.

Immediate programmatic open research questions are:

- What impact on transmission is achievable by actively detecting and eliminating all infections, including low-density malaria infections, using highly sensitive POC diagnostics in low transmission settings, particularly in areas of low vectorial capacity, compared to conventional malaria elimination methods (i.e., universal access to diagnosis and treatment and vector control), MDA, and active or reactive screen-and-treat campaigns using less sensitive POC diagnostics?
- In low and very low transmission settings, what is the proportion (or number) of infections that need to be detected and treated in order to accelerate the reduction of transmission towards malaria elimination?
- What is the cost–benefit for health systems in using highly sensitive diagnostics for specific target groups and in elimination settings? What are the most cost–effective deployment strategies for highly sensitive diagnostics in different settings?

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Annex 1: Outcome of the working groups and review by meeting participants

The working groups discussed several suggestions for updating the current WHO recommendation on the diagnosis of *P. falciparum* and *P. vivax* malaria in low transmission settings and the proposed changes are listed below.

Recommendation 1 (current wording):

Quality assured RDT and microscopy are the primary diagnostic tools for the confirmation and management of suspected clinical malaria in all epidemiological situations, including areas of low transmission, due to their high diagnostic performance in detecting clinical malaria, their wide availability and relatively low cost. Similarly, RDT and microscopy are appropriate tools for routine malaria surveillance (of clinical cases) in the majority of malaria-endemic settings.

Suggested changes:

- […] Similarly, conventional RDT and microscopy are appropriate tools for routine malaria surveillance (of clinical cases) in the majority of malaria-endemic settings. Malaria cases should be reported by type of diagnostic test used.

Recommendation 2 (current wording):

A number of nucleic acid amplification techniques are available and are more sensitive in detection of malaria compared to RDTs and microscopy. Generally, the use of more sensitive diagnostic tools should be considered only in low transmission settings where there is already widespread implementation of malaria diagnostic testing and treatment and low parasite prevalence rates (e.g., < 10%). Use of nucleic acid amplification (NAA)-based methods should not divert resources away from malaria prevention and control interventions and strengthening of the health care services, including the surveillance system.

Suggested changes:

- A number of highly sensitive techniques are available that detect low-density infections (below 100 parasites/µl). Generally, the use of more sensitive diagnostic tools should be considered only in low transmission settings where there is already widespread implementation of malaria diagnostic testing and treatment and low parasite prevalence rates (e.g. < 10%). Use of highly sensitive methods should not divert resources away from malaria prevention and control interventions and strengthening of the health care services, including the surveillance system.

Recommendation 3 (current wording):

Submicroscopic *Plasmodium falciparum* and *P. vivax* infections are common in low as well as high transmission settings. The use of NAA methods by malaria programmes should be considered for epidemiological research and surveys aimed at mapping submicroscopic infections at low transmission intensity. There may also be a use for NAA methods for identifying foci for special intervention measures in elimination settings.

Suggested changes:

- Low-density *Plasmodium falciparum* and *P. vivax* infections are found in low as well as high transmission settings. The use of highly sensitive tests by malaria programmes ~~should~~ may be considered for epidemiological research and surveys aimed at mapping low-density infections ~~submicroscopic infections at low transmission intensity~~. There may also be a use of highly sensitive methods for identifying foci for special intervention measures in elimination settings.

Recommendation 4 (current wording):

The majority of infections with asexual parasites have gametocytes detectable by molecular amplification methods, at low density not detectable by microscopy or RDTs. Most malaria infections (microscopic and submicroscopic) should be considered as potentially infectious and able to contribute to ongoing transmission. There is no need for routine detection of gametocytes using sensitive NAA methods in malaria surveys or clinical settings.

Suggested changes:

- […] Most malaria infections (including low-density infections ~~microscopic and submicroscopic~~) should be considered as potentially infectious, ~~and able to contribute to ongoing transmission,~~ but the extent of the contribution of low-density infections to transmission has yet to be determined. There is no need for routine detection of gametocytes using highly sensitive diagnostics ~~using sensitive NAA methods~~ in malaria surveys or clinical settings.

Recommendation 5

Common standards for nucleic acid based assays should be developed, including use of the WHO International Standard for *P. falciparum* DNA NAA assays and development of standards for other Plasmodium species, particularly *P. vivax* should be undertaken. A standard operating procedure should be developed which defines methods for sample collection, extraction, and the recommended equivalent quantity of blood to be added to the assay.

Development of an international, external quality assurance system is strongly recommended to ensure that data obtained from nucleic acid amplification assays are reliable and comparable.

Suggested addition:

- […] Reports presenting NAA results should include details of the methods used for sample collection and extraction, and the equivalent quantity of blood added for the PCR reaction, as well as details of outputs in DNA copies or parasite density.

Recommendation 6

In order to establish the role of serological assays in epidemiological assessments, there is a need for standardization and validation of reagents (antigens and controls), assay methodologies and analytical approaches.

Suggested changes:

No suggested changes.

Annex 2: List of pre-reads for the meeting

Main pre-reads:

1. Slater H, Okell L. Systematic literature review on the density, temporal dynamics and infectiousness of submicroscopic *P. falciparum* infections. Unpublished.

2. Robinson LJ, Hofmann NE, Karl S. The detectability and infectivity of submicroscopic Plasmodium *vivax* infections. Unpublished.

3. Kachur P. Clinical consequences of submicroscopic *P. vivax* and *P. falciparum* malaria infections. Unpublished.

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Additional suggested pre-reads:

8. Tripura R, Peto TJ, Veugen CC, Nguon C, Davoeung C, James N, et al. Submicroscopic Plasmodium prevalence in relation to malaria incidence in 20 villages in western Cambodia. Malar J. 2017;16(1):56. doi:10.1186/s12936-017-1703-5.

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19. Lin JT, Saunders DL, Meshnick SR. The role of submicroscopic parasitemia in malaria transmission: what is the evidence? Trends Parasitol. 2014;30(4):183–90. doi:10.1016/j.pt.2014.02.004.

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Outcome of the WHO Evidence Review Group on malaria low density infections

Dr A. Bosman

Malaria Policy Advisory Committee (MPAC) Meeting

17-19 October 2017

Chateau de Penthes, Pregny-Chambésy, Switzerland

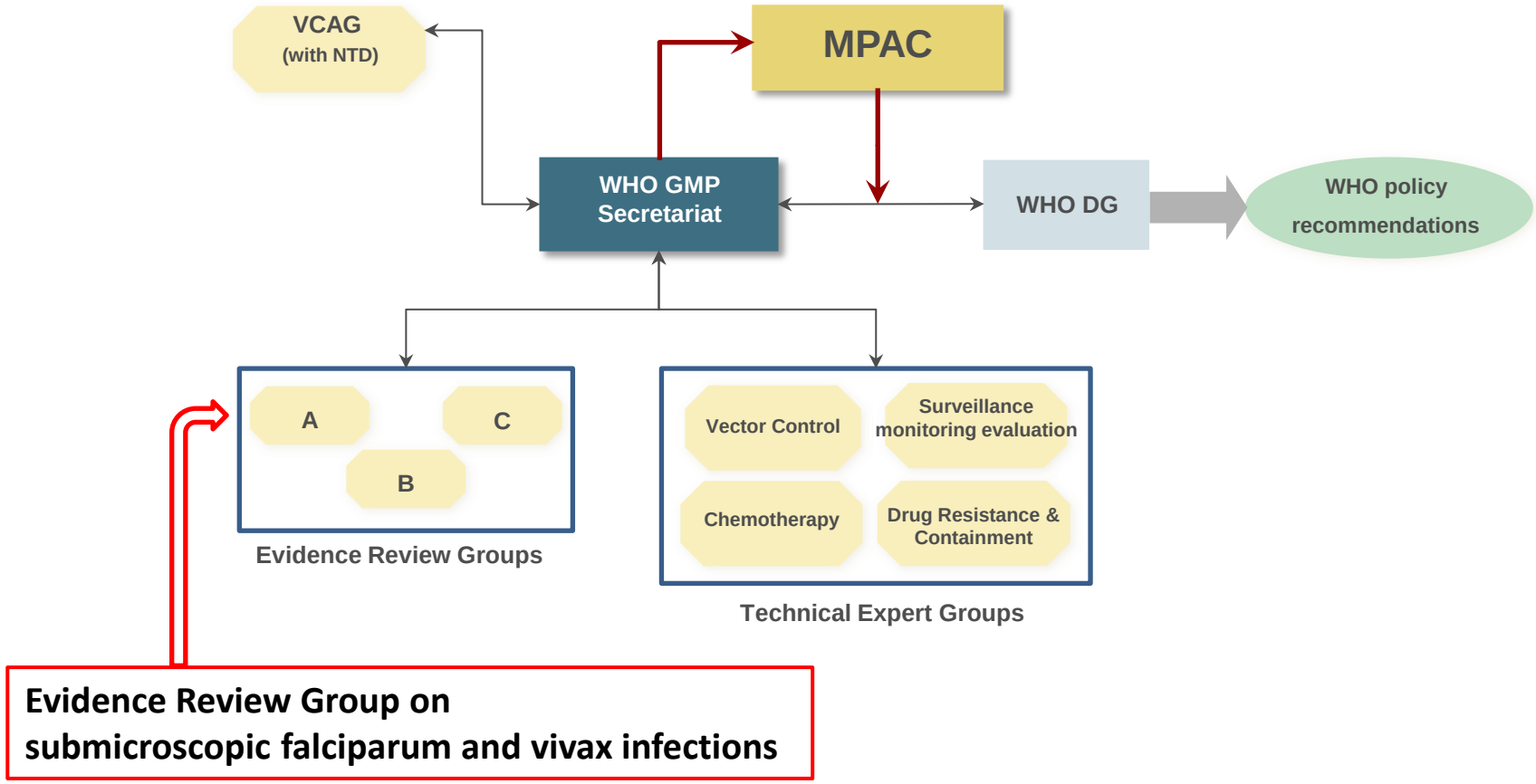
Global **Malaria** Programme

World Health Organization

Outline of the presentation

- WHO process for developing malaria policies
- ERG objectives, participants and method of work
- Conclusions of WHO ERG
- Draft recommendations for MPAC consideration

WHO policy-making process for malaria



Background of the ERG meeting

- In recent years, the application of nucleic acid amplification (NAA)-based diagnostic tools in epidemiological surveys and research has continued to expand.
- WHO reviewed the evidence in 2013 and issued recommendation on the use of malaria diagnostics in low transmission settings.
- A WHO evidence review group on MDA, MSAT and FSAT concluded in 2015 that using current point of care (POC) diagnostic tests, MSAT and FSAT are not suitable as interventions to interrupt malaria transmission.
- More recently, funding agencies, manufacturers and researchers have been working towards developing ultra-sensitive RDTs with limits of detection similar to those of NAA-based methods. One highly-sensitive RDT is now commercially available (Alere™ Malaria Ag P.f RDT, http://www.alere.com), with manufacturer claims of ten-fold higher sensitivity compared with conventional RDTs.
- The concept note, objectives and plan of convening a Evidence Review Group on submicroscopic falciparum and vivax infections were presented to the WHO Malaria Policy Advisory Committee in March 2017 and widely supported.

Objectives of the meeting

- To review data on the natural history of submicroscopic *P. falciparum* and *P. vivax* infections in different epidemiological settings, to evaluate implications for detectability, duration of infection, and infectivity, and to assess the relationship with symptoms of clinical malaria.
- To describe at population level the contribution of submicroscopic *P. falciparum* and *P. vivax* infections to transmission with respect to different levels of vectorial capacity and immunity in the population.
- To define procedures for the case management and reporting of submicroscopic *P. falciparum* and *P. vivax* infections identified through multiple means, e.g., reactive case detection, surveys, research, etc.
- To review and update the WHO recommendations on the diagnosis of *P. falciparum* and *P. vivax* malaria in low transmission settings, which were endorsed by the Malaria Policy Advisory Committee in March 2014, based on the report of the 2013 ERG meeting.
- To establish a set of research priorities and study design characteristics to address knowledge gaps on the relative importance of submicroscopic infections and the public health impact of detecting them using highly sensitive diagnostic tests.

Preparations for the meeting

- The GMP/PDT unit collaborated with Dr Teun Bousema, Radboud University Medical Center of The Netherlands, and Professor Chris Drakeley, London School of Tropical Medicine and Hygiene, in the planning of the ERG meeting, selection of studies and experts to prepare the literature reviews.
- Pre-reads of the meeting:

1. Hannah Slater & Lucy Okell: Systematic literature review on the density, temporal dynamics and infectiousness of submicroscopic *P. falciparum* infections
2. Leanne Robinson, Natalie Hofmann and Stephan Karl: The detectability and infectivity of submicroscopic *P. vivax* infections
3. Patrick Kachur: Clinical consequences of submicroscopic vivax and falciparum malaria infections
4. Gonçalves BP, Kapulu MC, Sawa P, Guelbéogo WM, Tiono AB, Grignard L, Stone W, Hellewell J, Lanke K, Bastiaens GJH, Bradley J, Nébié I, Ngoi JM, Oriango R, Mkabili D, Nyaurah M, Midega J, Wirth D, Marsh K, Churcher TS, Bejon P, Sirima SB, Drakeley C, Bousema T. The human infectious reservoir for *Plasmodium falciparum* malaria in areas of differing transmission intensity (submitted for publication)

List of participants

ERG Panel Members

- Graham BROWN (Chairperson)
- Sócrates HERRERA
- Patrick KACHUR
- Richard MAUDE
- Kamini MENDIS
- André Lin OUEDRAOGO
- Robert SINDEN
- Hannah SLATER
- Fitsum TADESSE

ERG Participants

- David BELL
- Teun BOUSEMA
- Gonzalo DOMINGO
- Chris DRAKELEY
- Jessica LIN
- Ivo MUELLER
- Lucy OKELL
- Leanne ROBINSON
- Thomas SMITH

According to WHO's Guidelines for Declaration of Interests (WHO expert), an interest is considered "personal" if it generates financial or non-financial gain to the expert, such as consulting income or a patent. "Specificity" states whether the declared interest is a subject matter of the meeting or work to be undertaken. An interest has "financial significance" if the honoraria, consultancy fee or other received funding, including those received by experts organization, from any single malaria-related company exceeds 10,000 USD in a calendar year. Likewise, a shareholding in any one malaria-related company in excess of 1,000 USD would also constitute a "significant shareholding".

List of participants (continued)

Rapporteur

- Natalie HOFMANN

Observers

- Jonathan COX
- Iveth GONZALEZ JIMENEZ

WHO Secretariat

- Pedro ALONSO
- Andrea BOSMAN
- Jane CUNNINGHAM
- Kimberly Ann LINDBLADE
- Abdisalan NOOR
- Peter OLUMESE
- David SCHELLENBERG
- P Silvia SCHWARTE

Conclusions of the ERG for MPAC review

1. A high proportion of *P. falciparum* and *P. vivax* infections identified in cross-sectional surveys are characterized by low parasite densities undetectable by conventional RDT and microscopy. Although limited by small sample sizes, the relative frequency of low-density infections appears to be higher in low transmission settings than in high transmission ones. The presence of such infections is likely influenced by many factors, including the recent history of transmission, rates of superinfection, genetic diversity of parasites, treatment and immunity. More detailed analyses of existing data and larger datasets from low to very low transmission settings are required in order to improve estimates of the proportion and distribution of low-density infections. Data are limited, and there is great uncertainty regarding estimates in very low transmission settings. More studies are required that also consider the recent history of transmission and potential impact of residual immunity in the population.

Proportion of low density infections

Table 1. The proportion of *P. falciparum* and *P. vivax* infections that are submicroscopic at different levels of transmission. Transmission intensity is classified by malaria prevalence assessed using NAA-based techniques. Data taken from published and unpublished studies that assessed *P. falciparum* and *P. vivax* parasitemia using NAA-based methods (Slater & Okell, Robinson, meeting pre-reads).

	Low transmission 0-10%	**Moderate transmission** 10-20%	**High transmission** >20%
P. falciparum			
Number of studies	n = 9	n = 1	n = 8
Unweighted Mean[1] (IQR)	75.0% (77.3 - 90.4)	not applicable	56.7% (51.4 - 63.6)
Weighted Mean[2] (CI_{95})	85.4% (81.5-88.7)	72.2 (67.4-76.6)	51.1% (48.7-53.5)
P. vivax			
Number of studies	n = 29	n = 20	n = 15
Unweighted Mean[1] (IQR)	82.5% (68.0-100)	72.6% (59.2-90.7)	57.2% (50.0-73.8)
Weighted Mean[2] (CI_{95})	70.7% (67.5-73.8)	72.0% (70.2-73.7)	58.1% (56.3-59.8)

[1] The unweighted mean is calculated by taking the raw average over all studies, by transmission level, of the proportion of submicropscopic infections observed in each study (independent of study size). The interquartile range is given as measure of variability in the proportion of submicropscopic infections between studies. For *P. falciparum* only one study was characterized as "moderate transmission" and the unweighted mean is thus not applicable.

[2] The weighted mean is calculated as an overall proportion of submicroscopic infections from accumulated data by transmission level and reported with a binomial 95% confidence interval.

Conclusions of the ERG for MPAC review

2. Evidence from several reports using mosquito-feeding experiments indicates that mosquitoes can be infected with low-density *P. falciparum* and *P. vivax* infections, although less efficiently than with high-density infections. For *P. vivax*, gametocyte densities closely follow those of asexual parasite stages. Transmission to mosquitoes becomes less efficient at *P. vivax* densities below the limit of detection (LOD) of expert microscopy (estimated at >10 parasites/µl), but can readily occur with infections below the LOD of field microscopy (estimated at >100 parasites/µl). For *P. falciparum*, the relation between gametocyte density transmissibility and the density of asexual parasitaemia is less predictable, and low-density infections below the detection level of expert microscopy can frequently result in mosquito infection. The outcome of experimental mosquito feeds is influenced by a variety of host, vector and parasite factors in addition to methodological factors, but their dynamic interactions are poorly understood.

Conclusions of the ERG for MPAC review

3. Depending on the relative proportions of low- and high-density infections in a particular location, the role of each in overall transmission may vary considerably. Mosquito feeding experiments help to measure the infectiousness of low- and high-density infections for mosquitoes. However, there are limited data on the relative contributions of low- and high-density *P. falciparum* and *P. vivax* infections to the onward transmission to human populations at the community level. It is critically important to understand the contribution of low-density infections to malaria transmission in order to inform effective malaria control strategies.

4. Conclusive data on the natural history of low-density *P. falciparum* and *P. vivax* infections in different endemic settings remain elusive. Knowledge gaps exist in understanding the longitudinal dynamics of parasite density and infectivity in untreated chronic natural infections; identifying risk factors for carriage of low-density infections; and understanding the prospective clinical and pathological impacts of untreated low-density infections. Available evidence related to the different parasite biology of *P. falciparum* and *P. vivax* suggests that chronicity of infection is achieved through different mechanisms for the two species: antigenic variation and persistence in the blood stream for *P. falciparum*, and periodical relapses for *P. vivax*.

Blood stage infection dynamics

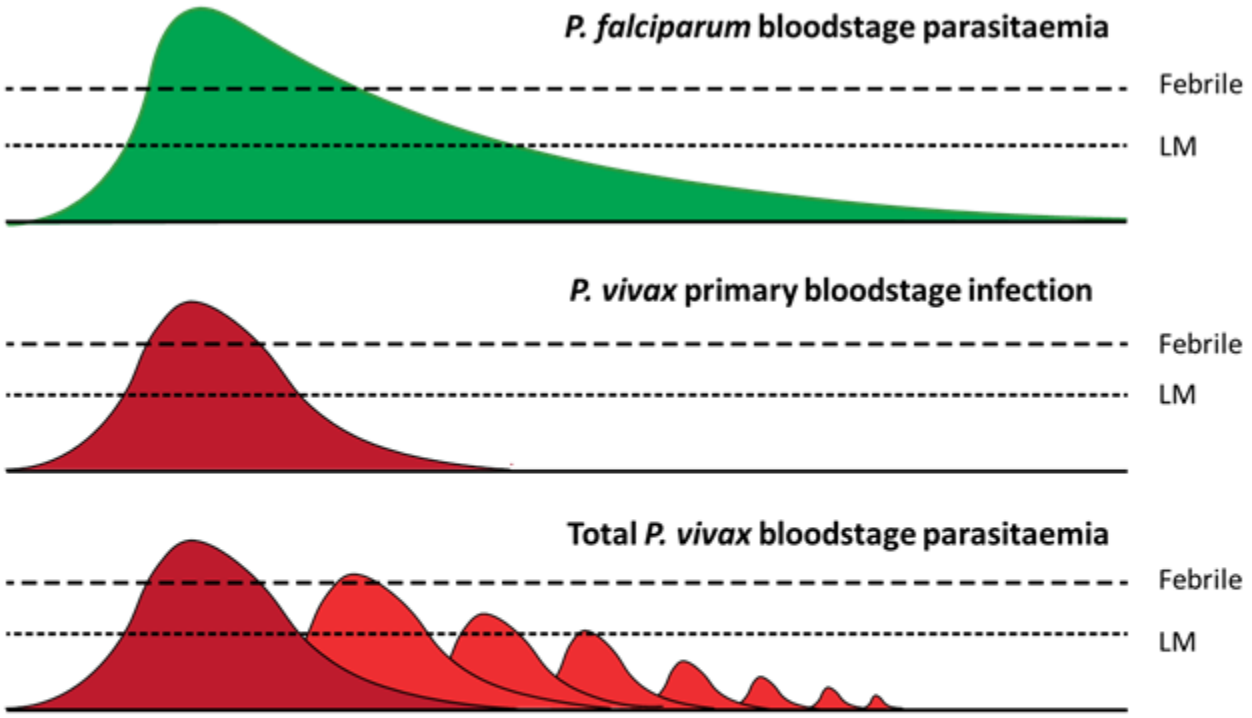


Figure 2. Models of the average pattern of *P. falciparum* and *P. vivax* blood-stage infection dynamics. Blood stage parasitemia is depicted in the absence of super-infections. Within individual infections, there is fluctuation in density. Figure taken from the ERG presentation by Ivo Mueller.

Conclusions of the ERG for MPAC review

5. With the available evidence, it is difficult to accurately predict how the identification and treatment of low-density *P. falciparum* and *P. vivax* infections through active screen-and-treat based interventions in different endemic settings would impact transmission. Moreover, it is not possible to predict the proportion of the total infectious reservoir that would need to be detected and eliminated in order to accelerate the reduction of transmission. Intervention trials in different epidemiological settings using appropriate control interventions are warranted in order to evaluate the impact on transmission and cost–benefit of applying highly sensitive diagnostics for targeting low-density infections. Until the outcomes of such trials are available, highly sensitive diagnostics should not be part of any routine malaria control or elimination programme; their use should be limited to research purposes.

Detectability of low parasite infections

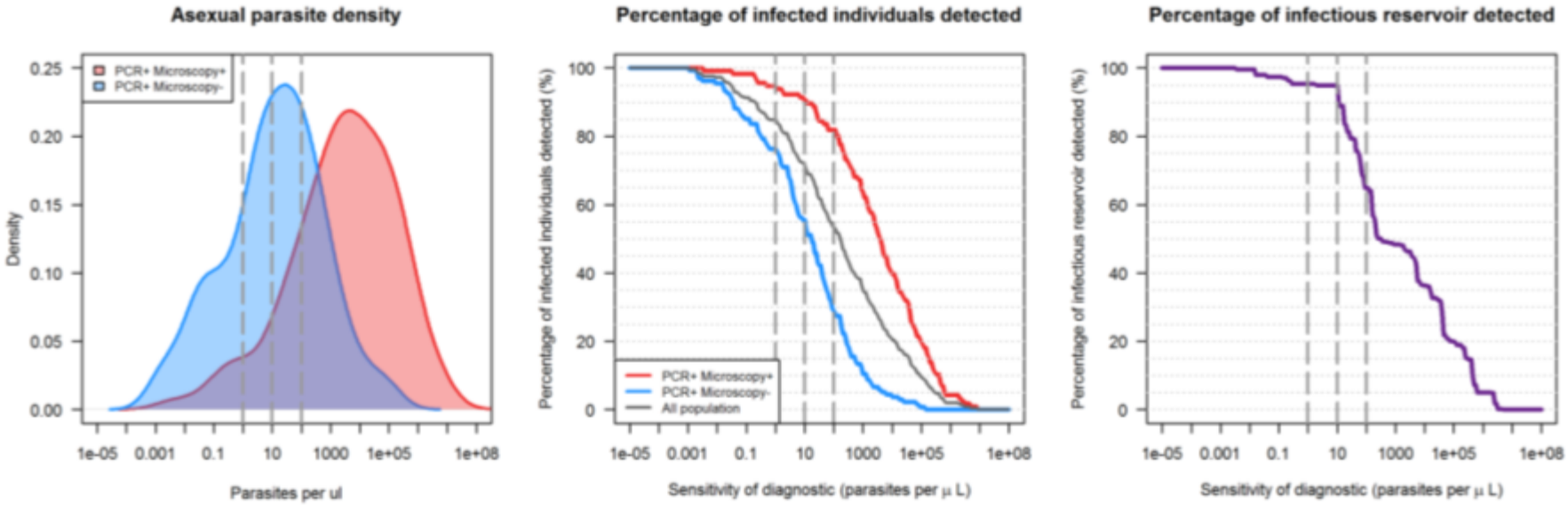


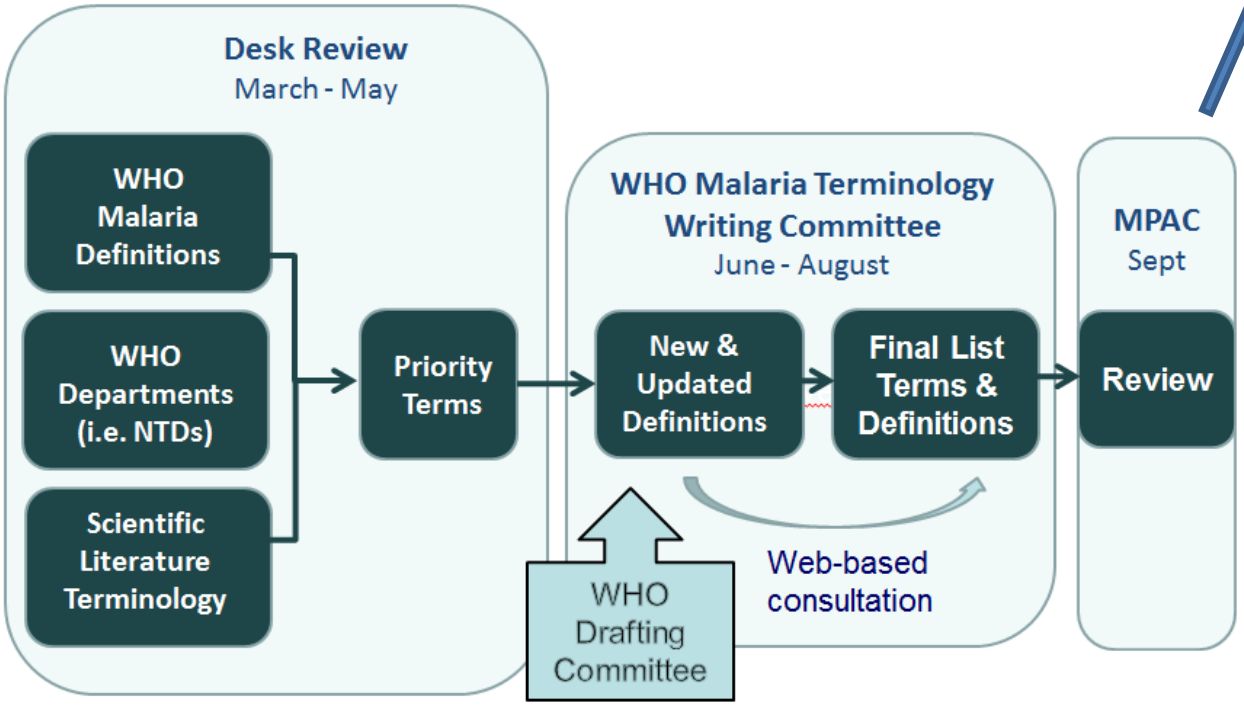
Figure 3. Parasite density distributions by quantitative NAA methods of individuals from Burkina Faso that are detectable (red) and undetectable (blue) by microscopy (left panel); and the proportion of infected individuals (middle panel) and of the infectious reservoir (right panel) that is detected with different diagnostic sensitivity thresholds (1, 10 and 100 parasites/µl). Figure from Slater & Okell, meeting pre-read, unpublished data.

Conclusions of the ERG for MPAC review

6. To improve comparability of results, better harmonization and standardization is required in the reporting of the molecular methods used for the detection, identification and quantification of malaria parasites in epidemiological surveys and research studies. Adherence to the Minimum Information for Publication of Quantitative Real-time PCR Experiments (MIQE) guidelines for reporting quantitative PCR results, as well as the validation of nucleic acid-based amplification assays using standardized and quality controlled material (such as the WHO International Standard for P. falciparum DNA NAA Assays) is strongly encouraged. Until standardization is achieved, all reports should include a detailed description of the precise methods used to obtain the data being reported, including the analytical sensitivity and specificity of tests.

Additional ERG expected outcome

- Agrement on term = submicroscopic infection or low density parasitemia or subpatent infection or ... ?
- Agreement on definition = blood stage parasitaemia below XX parasites/µL excluding isolated gametocytemia or … ?



Conclusions of the ERG for MPAC review

7. The terms “submicroscopic,” “asymptomatic,” and “low-density” infection are often used interchangeably in the literature, generating confusion. “Submicroscopic” generally implies parasitaemia that is below the LOD of microscopy or RDT, but detectable using molecular or other highly sensitive diagnostic methods. The use of the term “submicroscopic” for describing low-density malaria infections should be discouraged. The term “asymptomatic” is not based on parasite density and instead refers to the absence of signs and symptoms of malaria. Asympomatic malaria should be defined with respect to the absence of specific clinical manifestations and the time period evaluated in relation to infection detection. In light of these definitions, the term “low-density” infection is considered most appropriate. When parasitaemia is quantified, a clear definition of “low-density infection” should be reported (suggested: <100 parasites/µl), accompanied by a description of the method of quantification. In studies that do not quantify parasitaemia, low-density infections can be defined as those identified through highly sensitive methods but not detected using conventional diagnostics (microscopy or RDT).

Conclusions of the ERG for MPAC review

8. Updating the WHO recommendations on the diagnosis of *P. falciparum* and *P. vivax* malaria in low transmission settings is required in order to clarify that WHO does not currently recommend highly sensitive RDTs, other highly sensitive non-NAA-based methods, or NAA-based methods for parasite detection in the routine management of clinical malaria and surveillance. Research is needed to document the public health benefits and cost–effectiveness of detecting and treating low-density infections in low transmission areas and/or specific population groups. In particular, potential research objectives for highly sensitive diagnostics could include epidemiological research to understand the contribution of low-density infections to transmission, border screening of immigrants or migrant populations, foci investigations including the mapping of low-density infections, and use in pregnant women for the detection and treatment of low or sequestered parasite biomass.

Draft recommendations for MPAC review

To comply with the above conclusions, the WHO/GMP secretariat in consultation with the ERG Panel Members developed draft recommendations on the diagnosis of *P. falciparum* and *P. vivax* malaria in low transmission settings. These are listed below for consideration by the WHO MPAC.

8.1. Quality-assured conventional RDT and microscopy are the recommended diagnostic tools for the confirmation and management of malaria cases and malaria surveillance, including routine health information systems and household surveys, in all epidemiological situations. Malaria cases should be reported by type of diagnostic test used.

Draft recommendations for MPAC review

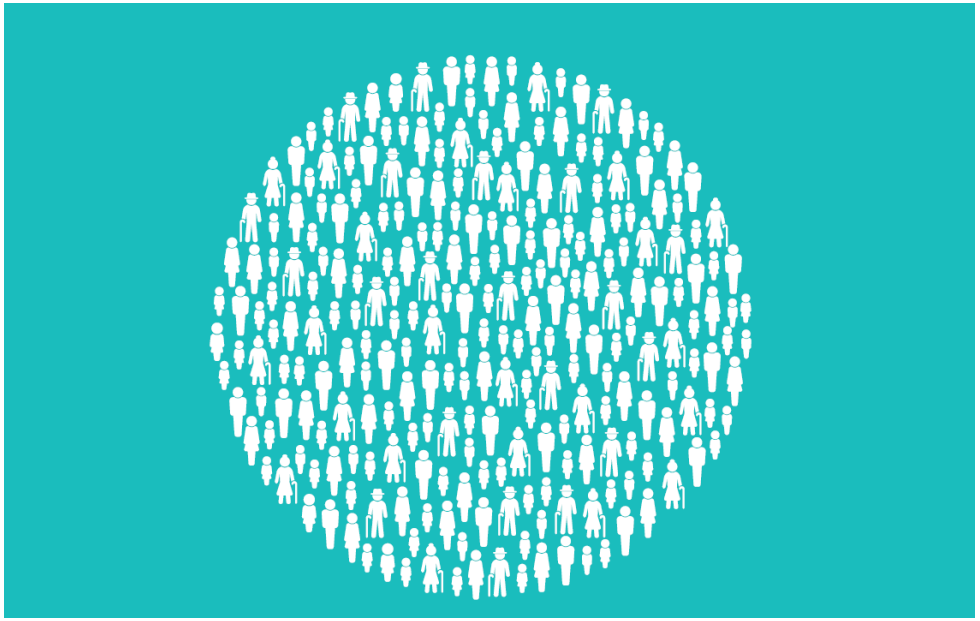
8.2. A number of highly sensitive techniques are available that detect low-density infections (below 100 parasites/µl). Until there is evidence that the detection of low-density infections using these tools will accelerate malaria elimination, in elimination settings, these tools should only be used for research purposes.

8.3. The majority of infections with asexual parasites have gametocytes detectable by NAA methods, and there is no known benefit of routine detection of low-density gametocytes by molecular methods. All malaria infections (including those infections with low-density parasitaemia) should be considered as potentially infectious.

8.4. Presentation of NAA results should include details of the methods used for sample collection and extraction, and the equivalent quantity of blood added for the PCR reaction, as well as details of outputs in DNA copies or parasite density.

8.5. Before the role of serological assays in malaria elimination programmes can be determined, there is a need for standardization and validation of reagents (antigens and controls), assay methodologies and analytical approaches.

Many thanks
for your kind attention



Outcomes from Evidence Review Group on Deployment of Pyrethroid-PBO Nets

Malaria Policy Advisory Group Meeting

Geneva, Switzerland

17 October 2017

Global **Malaria** Programme

World Health Organization

Background

- Several mosquito nets that include both a pyrethroid insecticide and the synergist piperonyl butoxide (PBO) have become available
- PBO is a synergist that inhibits metabolic enzymes within the mosquito that detoxify or sequester insecticides
- Compared to a pyrethroid-only net, a pyrethroid-PBO net should, in theory, have an increased killing effect on malaria vectors that express metabolic resistance mechanisms
- 5 pyrethroid-PBO nets evaluated under WHOPES to determine whether they met the criteria established for classification as a LLIN (PBO component not evaluated)
- All 5 pyrethroid-PBO nets underwent experimental hut evaluations, and two are currently undergoing long-term field evaluations
- In 2015, WHO's Global Malaria Programme (GMP) convened the first Evidence Review Group to define conditions for use of pyrethroid-PBO nets

Summary of 2015 ERG recommendations (1)

1. Evidence is still limited to justify a complete switch from pyrethroid-only LLINs to PBO LLINs across all settings
2. PBO LLINs with a WHOPES interim or full recommendation considered to be at least an equivalent option to other LLINs in all settings, and probably superior in some settings. However, neither evidence to assume their higher efficacy nor greater utility in a resistance management strategy in all settings.
3. PBO LLINs should be used only where universal coverage with effective vector control will not be reduced
4. Due to the potential for an antagonistic effect between PBO and organophosphates, PBO LLINs should not be used in areas programmed for IRS with pirimiphos-methyl CS

Summary of 2015 ERG recommendations (2)

5. To build evidence base to support deployment of PBO LLINs, pilot "exploratory" implementation is necessary. Pilot implementation not to be undertaken unless accompanied by robust evaluation
6. To guide potential deployment of PBO LLINs, countries considering pilot exploratory implementation should:
    - collect data on the presence, level, intensity and mechanisms of resistance to all insecticide classes;
    - design an evaluation with appropriate indicators
7. To manage insecticide resistance, WHO urgently calls for the development and evaluation of non-pyrethroid LLINs and other innovative vector control tools for use across all settings.

These recommendations will be revised periodically, on the basis of emerging evidence.

2017 ERG objectives

ERG reconvened from 26-27 June 2017 to:

- Review new evidence from a cluster randomized controlled trial in Muleba, Tanzania on the comparative impact of a pyrethroid-PBO net when compared to a pyrethroid-only LLIN
- Review new evidence, where available on:
  - LLIN chemical content and bio-availability of pyrethroids and PBO following standard WHO laboratory washing.
  - Efficacy of pyrethroid-PBO nets when compared to pyrethroid-only LLINs in experimental hut trials or entomological evaluations.
  - the possibility of predicting epidemiological outcomes based on entomological data through an epidemiological modelling approach.
- Consider whether 2015 recommendations for deployment of pyrethroid-PBO nets should be updated
- Consider whether existing guidance on how to evaluate and monitor the impact / effectiveness of pyrethroid-PBO nets requires updating

Tanzania trial

Muleba trial site

- Rural district western shore of lake Victoria
- Malaria prevalence 40% in children 6 months to 14 yrs
- 2 transmission seasons: Nov/Dec and Jun/Jul
- Confirmed insecticide resistance

- Four arm cluster randomised factorial design, 12 clusters per arm. Control arm = pyrethroid-only LLIN (Olyset)
- Data are confidential until published

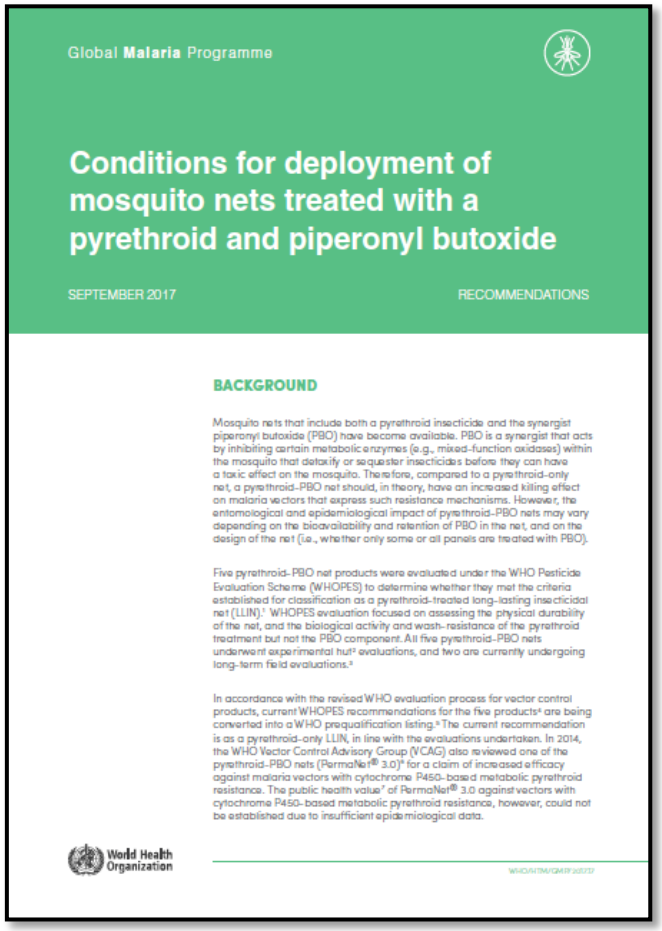
ERG conclusions & recommendations

1. Epidemiological data from one cluster randomized controlled trial suggest that pyrethroid-PBO net products may have additional public health value when compared to pyrethroid-only LLIN products in areas where the main malaria vector has confirmed moderate levels of pyrethroid resistance
2. Based on the epidemiological findings and the need to deploy products that are effective against pyrethroid-resistant mosquitoes, pyrethroid-PBO nets are being given an interim endorsement as a new WHO class of vector control products
3. National malaria control programmes and their partners should consider deployment of pyrethroid-PBO nets in areas where pyrethroid resistance has been confirmed in the main malaria vectors

ERG conclusions & recommendations

4. Further evidence on pyrethroid-PBO nets is required to support the refinement of WHO guidance regarding the conditions for the deployment of products in this class:
    - Review evidence from Y3 of Tanzania trial
    - Review evidence from additional trials (Uganda) to determine performance in a different geographical setting and identify any notable differences in effectiveness between products in this class. If additional public health value confirmed it will allow interim endorsement of pyrethroid-PBO nets to be converted into full establishment of the class
    - Identify methods to determine if other products in class perform as well as product for which epi data generated
    - Further investigate potential antagonistic effects with pirimiphos-methyl
    - Further research relationship between ento indices and epi outcomes
    - Validate synergist testing doses and methods
5. Pyrethroid-PBO nets should not be considered a tool that can effectively manage insecticide resistance in malaria vectors

Further information



Global **Malaria** Programme

Conditions for deployment of mosquito nets treated with a pyrethroid and piperonyl butoxide

SEPTEMBER 2017 RECOMMENDATIONS

BACKGROUND

Mosquito nets that include both a pyrethroid insecticide and the synergist piperonyl butoxide (PBO) have become available. PBO is a synergist that acts by inhibiting certain metabolic enzymes (e.g., mixed-function oxidases) within the mosquito that detoxify or sequester insecticides before they can have a toxic effect on the mosquito. Therefore, compared to a pyrethroid-only net, a pyrethroid-PBO net should, in theory, have an increased killing effect on malaria vectors that express such resistance mechanisms. However, the entomological and epidemiological impact of pyrethroid-PBO nets may vary depending on the bioavailability and retention of PBO in the net, and on the design of the net (i.e., whether only some or all panels are treated with PBO).

Five pyrethroid-PBO net products were evaluated under the WHO Pesticide Evaluation Scheme (WHOPES) to determine whether they met the criteria established for classification as a pyrethroid-treated long-lasting insecticidal net (LLIN).[1] WHOPES evaluation focused on assessing the physical durability of the net, and the biological activity and wash-resistance of the pyrethroid treatment but not the PBO component. All five pyrethroid-PBO nets underwent experimental hut[2] evaluations, and two are currently undergoing long-term field evaluations.[3]

In accordance with the revised WHO evaluation process for vector control products, current WHOPES recommendations for the five products[4] are being converted into a WHO prequalification listing.[5] The current recommendation is as a pyrethroid-only LLIN, in line with the evaluations undertaken. In 2014, the WHO Vector Control Advisory Group (VCAG) also reviewed one of the pyrethroid-PBO nets (PermaNet® 3.0)[6] for a claim of increased efficacy against malaria vectors with cytochrome P450- based metabolic pyrethroid resistance. The public health value[7] of PermaNet® 3.0 against vectors with cytochrome P450- based metabolic pyrethroid resistance, however, could not be established due to insufficient epidemiological data.

World Health Organization

http://www.who.int/malaria/publications/atoz/use-of-pbo-treated-llins/en/

Current & next steps

- Transition from WHOPES to revised evaluation system for vector control products. Need to provide clarity on:
  - what vector control products have been evaluated for by WHOPES (and what they have not been evaluated for)
  - any data needs associated with evaluation under the revised system, and to allow formulation of programmatic guidance
- Five PBO nets are currently WHOPES recommended as pyrethroid-only nets (scope of current recommendations may not have been clear)
- Transition to revised evaluation system does not intend to withdraw existing WHO recommendations, but to build on these

Current & next steps

- Existing WHOPES recommendations will be converted to PQ ‘listing’; this provides a means to communicate details on each product’s status
- To complete assessment of the class and provide more detailed programmatic guidance, WHO will require:
  - For Olyset Plus, a second epidemiological trial to assess public health value in a different geographical setting
  - For all nets, additional data to assess bioavailability and chemical retention of PBO
  - For each nets other than Olyset Plus, demonstration that it is of non-inferior entomological performance. Details on how this will be assessed are being worked on.

Conditions for deployment of mosquito nets treated with a pyrethroid and piperonyl butoxide

SEPTEMBER 2017 (REVISED DECEMBER 2017) RECOMMENDATIONS

BACKGROUND

Mosquito nets that include both a pyrethroid insecticide and the synergist piperonyl butoxide (PBO) have become available. PBO is a synergist that acts by inhibiting certain metabolic enzymes (e.g., mixed-function oxidases) within the mosquito that detoxify or sequester insecticides before they can have a toxic effect on the mosquito. Therefore, compared to a pyrethroid-only net, a pyrethroid-PBO net should, in theory, have an increased killing effect on malaria vectors that express such resistance mechanisms. However, the entomological and epidemiological impact of pyrethroid-PBO nets may vary depending on the bioavailability and retention of PBO in the net, and on the design of the net (i.e., whether only some or all panels are treated with PBO).

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In accordance with the revised WHO evaluation process for vector control products, current WHOPES recommendations for the five products[4] are being converted into a WHO prequalification listing.[5] In line with the evaluations undertaken, the WHO recommendation for these products has been as pyrethroid-only LLINs. In 2014, the WHO Vector Control Advisory Group (VCAG) also reviewed one of the pyrethroid-PBO nets (PermaNet® 3.0)[6] for a claim of increased efficacy against malaria vectors with cytochrome P450-based metabolic pyrethroid resistance. The public health value[7] of PermaNet® 3.0 against vectors with cytochrome P450-based metabolic pyrethroid resistance, however, could not be established due to insufficient epidemiological data.

World Health Organization

WHO/HTM/GMP/2017.17

In 2015, WHO's Global Malaria Programme (GMP) convened an Evidence Review Group (ERG) to define the conditions for use of pyrethroid-PBO nets. WHO released an initial set of recommendations in December 2015. Since the 2015 ERG, a randomized controlled trial in the United Republic of Tanzania has generated new epidemiological evidence for pyrethroid-PBO nets. As a result, the WHO/GMP ERG re-convened in June 2017 to assess whether these new data demonstrate the public health value of pyrethroid-PBO nets in terms of the control of malaria where vectors are pyrethroid-resistant. Details of the review process, quality of the evidence, outstanding questions, and proposals to further strengthen the current evidence can be found in the ERG meeting report, which will be made available upon publication of the randomized control trial data.[8]

In the ongoing transition of the WHO evaluation process for vector control products from WHOPES to the Prequalification Team, WHO has developed an updated policy recommendation on pyrethroid-PBO nets that takes into account the epidemiological trial data from Tanzania. This update is an attempt to further clarify the available evidence base for these types of nets, their categorization under the revised evaluation system, and the additional data required to support WHO's policy-making process. This represents an exception to the standard review procedure, which requires a minimum of two epidemiological trials to assess the public health value of new vector control tools not covered by an existing WHO policy.

These recommendations replace the 2015 WHO recommendations on pyrethroid-PBO nets and will be further revised as new data become available.

CONCLUSIONS & RECOMMENDATIONS

On the basis of the current evidence, WHO concludes and recommends the following:

1. **Epidemiological data from one cluster randomized controlled trial indicated that a pyrethroid-PBO net product had additional public health value compared to a pyrethroid-only LLIN product in an area where the main malaria vector had confirmed pyrethroid resistance of moderate intensity conferred (at least in part) by monooxygenase-based resistance mechanism as determined by standard procedures.**[9, 10] This conclusion is based on a comparison of malaria infection rates in children in village clusters allocated pyrethroid-PBO nets (Olyset® Plus) and rates in village clusters allocated pyrethroid-only LLINs (Olyset® Net) over a period of 2 years in Muleba, United Republic of Tanzania. Entomological data from experimental hut studies on several similar pyrethroid-PBO products conducted in areas of pyrethroid resistance support the finding that pyrethroid-PBO nets are more effective at killing resistant mosquitoes. Mathematical modelling work drawing on relevant entomological data indicates that the added benefit of pyrethroid-PBO nets compared to pyrethroid-only LLINs is expected to be the greatest where pyrethroid resistance is at "intermediate levels", meaning where mosquito mortality after exposure to a pyrethroid insecticide in WHO test kits or CDC bottle assays ranges from 10% to 80%.[11] The benefit of pyrethroid-PBO nets is expected to diminish where bioassay mortality is outside of this range. Pyrethroid-PBO nets are not expected to have any added benefit in areas where the main malaria vectors are susceptible to pyrethroids and/or do not harbor resistance mechanism(s) that are affected by PBO, i.e., monooxygenase-based resistance mechanism.[10]

2. **Based on the epidemiological findings and the need to deploy products that are effective against pyrethroid-resistant mosquitoes, pyrethroid-PBO nets are being given a conditional endorsement as a new WHO class of vector control products.** As an exception, this establishment of a class is based on a single epidemiological study instead of two studies, as required by VCAG for the assessment of a new product class.[5] The endorsement is based on epidemiological evidence of the greater effectiveness of pyrethroid-PBO nets in areas of intermediate level resistance. Full confirmation of the class will require VCAG's assessment of data from a second epidemiological trial. Meanwhile, all pyrethroid-PBO nets that have a WHOPES recommendation or WHO prequalification listing will be considered to be at least as effective as pyrethroid-only LLINs at preventing malaria infections – and possibly more effective in areas with intermediate levels of pyrethroid resistance conferred by a monooxygenase-based resistance mechanism.

3. **National malaria control programmes and their partners should consider the deployment of pyrethroid-PBO nets in areas where the main malaria vector(s) have pyrethroid resistance that is: a) confirmed, b) of intermediate level (as defined above), and c) conferred (at least in part) by a monooxygenase-based resistance mechanism, as determined by standard procedures.**[10] Deployment of pyrethroid-PBO nets must only be considered in situations where coverage with effective vector control (primarily LLINs or indoor residual spraying [IRS]) will not be reduced; the primary goal must remain the achievement and maintenance of universal coverage for all people at risk of malaria.

4. **Further evidence on pyrethroid-PBO nets is required to support the refinement of WHO guidance regarding conditions for the deployment of products in this class:**

    a. VCAG will review data from the third intervention year of the ongoing randomized control trial in Tanzania once they become available. This will determine whether the higher effectiveness of the pyrethroid-PBO net (compared to a pyrethroid-only LLIN) has continued to be observed over the full period for which an LLIN is expected to retain its biological activity (i.e., a minimum of 3 years). These data will contribute to our understanding of whether the pyrethroid-PBO product under evaluation meets the former WHOPES requirements for an LLIN.

    b. VCAG will review further epidemiological trial data as soon as they become available, such as from a randomized controlled trial planned in Uganda using two pyrethroid-PBO nets (the same product as is being tested in Tanzania, treated with PBO on all panels, and another pyrethroid-PBO net with only the net roof treated with PBO). These data will provide additional evidence on how pyrethroid-PBO nets perform in another geographical setting and whether there are notable differences in effectiveness between products in this class. If VCAG is able to confirm additional public health value, it will allow the conditional endorsement of pyrethroid-PBO nets to be converted into the full establishment of the class.

    c. The effectiveness of other pyrethroid-PBO nets in comparison to the product for which data were generated in Tanzania needs to be determined. Evaluation procedures to determine whether other

products in a class perform at least as well as the product(s) for which epidemiological data were generated, and for which a product class has been established, are under development. Comparing the effectiveness of different pyrethroid-PBO nets will be aided by:

c.i. Identifying appropriate entomological indicators to assess the effectiveness of subsequent products entering an existing product class, given that these products will not be required to generate epidemiological data;

c.ii. Conducting comparative experimental hut trials on different pyrethroid-PBO nets to determine the relative effectiveness of different compositions of net (e.g., PBO applied to the roof panel of the net only versus all panels of the net), as well as different formulations including initial PBO treatment dosages and release properties;

c.iii. Conducting bioassays using characterized reference strains of insecticide-resistant *Anopheles* mosquito(es) on pyrethroid-PBO nets following a minimum of 2 to 3 years of routine use to determine the bioavailability and chemical retention of PBO over time. Current information suggests that PBO retention rates and wash resistance indices are much lower than for the pyrethroid component of the formulations. Studies should be conducted on the PBO-LLIN product assessed in Tanzania, with comparative studies performed on other products of the same class.

d. Further investigations (laboratory and field studies) are required to determine if there is an antagonistic effect between PBO and the organophosphate pirimiphos-methyl, which is one insecticide recommended for IRS. To date, limited evidence from laboratory studies and the randomized controlled trial in Tanzania suggests that this is not an operational concern; however, further studies are needed to determine the generalizability of current findings.

e. Further research will be required to investigate the relationship between entomological indices and epidemiological outcomes for vector control products in order to determine whether entomological surrogates may be sufficient for assessing the public health value of vector control products not currently covered by a WHO policy recommendation.

f. Synergist testing methods need to be validated, including identification of appropriate sub-lethal concentrations for pre-exposure to PBO in CDC bottle assays.

5. **Pyrethroid-PBO nets should not be considered a tool that can effectively manage insecticide resistance in malaria vectors.** It is an urgent task to develop and evaluate LLINs treated with non-pyrethroid insecticides and other innovative vector control tools for use across all settings in order to provide alternatives for use in a comprehensive insecticide-resistance management strategy.

Endnotes

The mention of specific companies or certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.

1. Guidelines for laboratory and field testing of long-lasting insecticidal nets. Geneva: World Health Organization; 2013 (http://apps.who.int/iris/bitstream/10665/80270/1/9789241505277_eng.pdf).

2. Phase II WHOPES evaluation

3. Phase III WHOPES evaluation

4. WHO recommended long-lasting insecticidal nets. Geneva: World Health Organization; 2017 (http://who.int/whopes/Long-lasting_insecticidal_nets_June_2017.pdf).

5. The evaluation process for vector control products. Geneva: World Health Organization; 2017 (http://www.who.int/malaria/publications/atoz/evaluation-process-vector-control-products/).

6. Second meeting of the Vector Control Advisory Group. Summary report. Geneva: World Health Organization; 2014 (http://apps. who.int/iris/bitstream/10665/137318/1/9789241508025_eng.pdf).

7. Public health value is defined as: proven protective efficacy to reduce or prevent infection and/or disease in humans.

8. Report of the evidence review group to define the conditions of deployment of mosquito nets treated with a pyrethroid and piperonyl butoxide, 26-27 June 2017. Geneva: World Health Organization; 2017. This will only be available when the data from Tanzania have been published.

9. Protopopoff N & Rowland M. Effectiveness of a long-lasting PBO treated insecticidal net and indoor residual spray interventions, separately and together, against malaria transmitted by pyrethroid resistant mosquitoes: A community randomised factorial design trial.(Under final peer review)

10. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes (2nd edition). Geneva: World Health Organization; 2016 (http://www.who.int/malaria/publications/atoz/9789241511575/).

11. Intermediate level of resistance classified by epidemiological predictions of pyrethroid-PBO nets on average averting >0.1 clinical cases per person per year over pyrethroid-only LLINs (Churcher TS, Lissenden N, Griffin JT, Worrall E, Ranson H. The impact of pyrethroid resistance on the efficacy and effectiveness of bednets for malaria control in Africa. Elife. 2016;5:e16090 [https://elifesciences.org/articles/16090]).

Update on malaria elimination in the Greater Mekong Subregion

Dr. Rabi Abeyasinghe/WPRO, Dr. Eva Christophel/SEARO,
Dr. Hiro Okayasu/MME

World Health Organization

Outline

Background

Trend

Strategy

MME Team and Support

Map of TES Sites

Pharma support

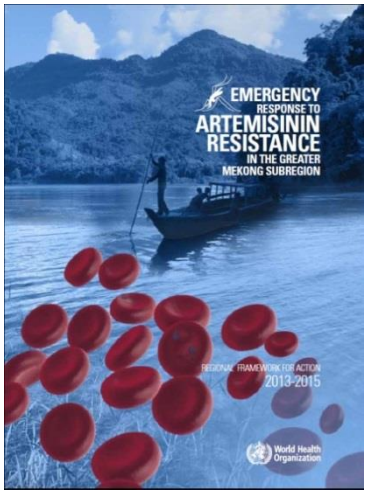
Regional Coordination

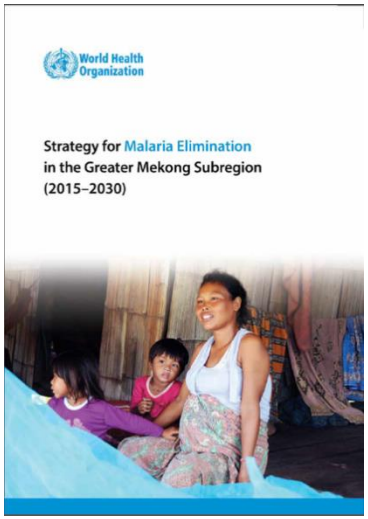
Country Progress

Challenges

World Health Organization

Background

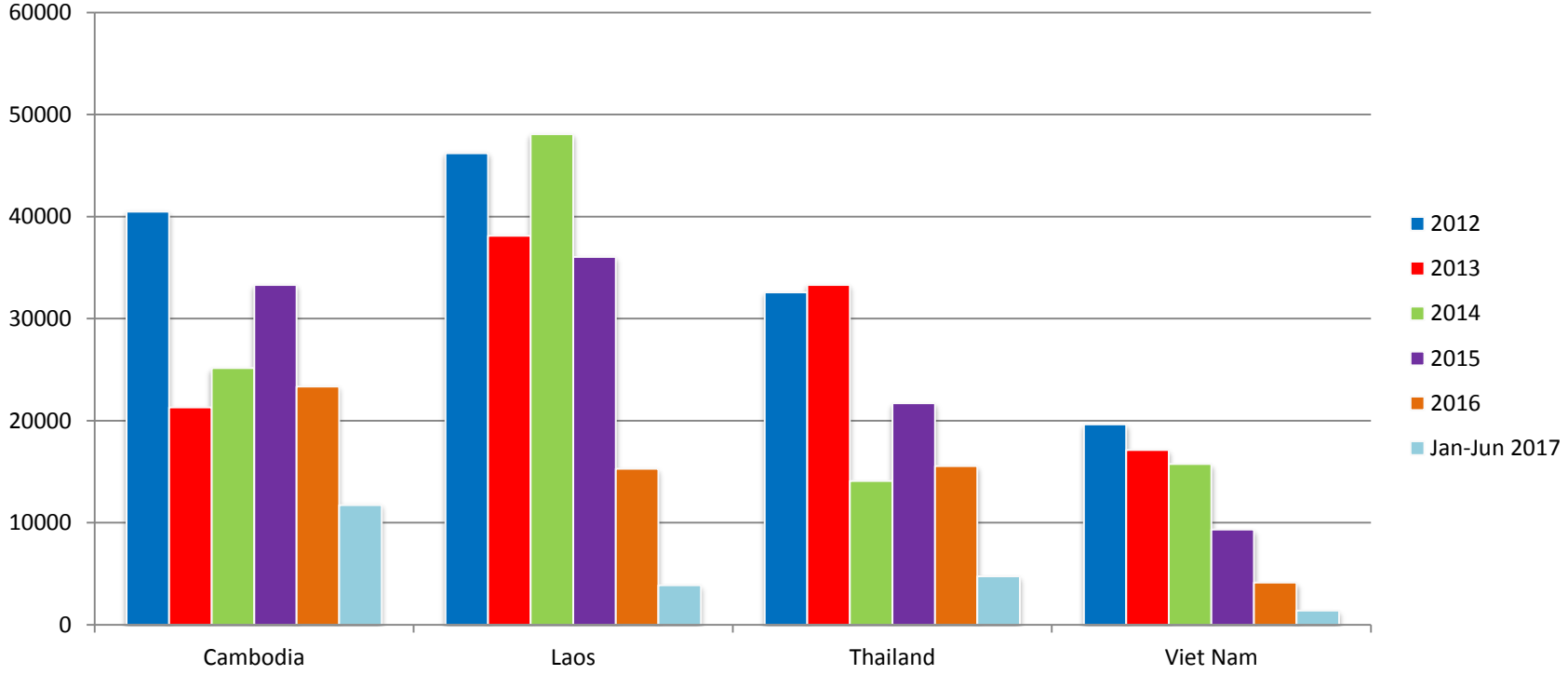




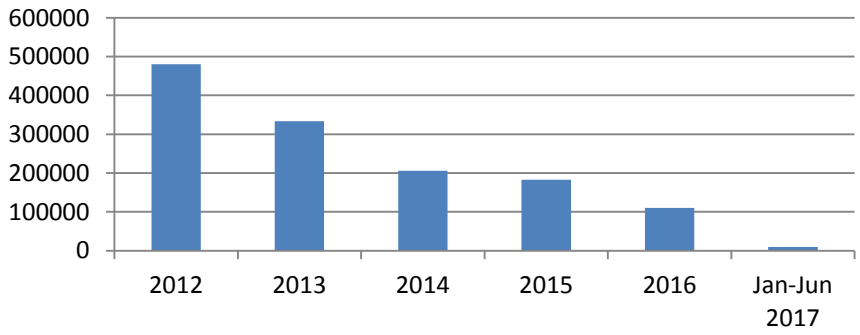
- Since 2008: Artemisinin resistance containment and elimination
  - Thailand and Cambodia border
- April 2013: WHO launched ERAR framework for GMS
  - To contain artemisinin resistance in the GMS
  - ERAR established as regional hub to coordinate containment strategies
- MPAC, Sep 2014: Elimination of *P. falciparum* in the GMS by 2030
- WHA, 2015: Strategy for malaria elimination in the GMS (2015–2030)
- 2016: Transitional year for the ERAR hub
  - Support national strategic plans to accelerate towards elimination
- 2017 onwards: GMS Malaria Elimination Efforts coordinated from Cambodia and implemented through strengthened country offices (backed by Regional Offices and GMP) supporting national efforts.
  - MME Coordinator Dr Hiromasa Okayasu in place since August 2017.

World Health Organization

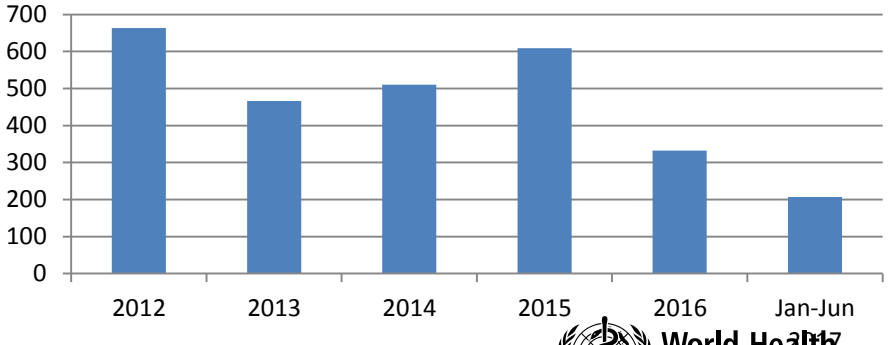
Malaria case reports in GMS from regional data sharing platform [2012- June 17]



Myanmar Confirmed malaria Cases (2012-June 2017)

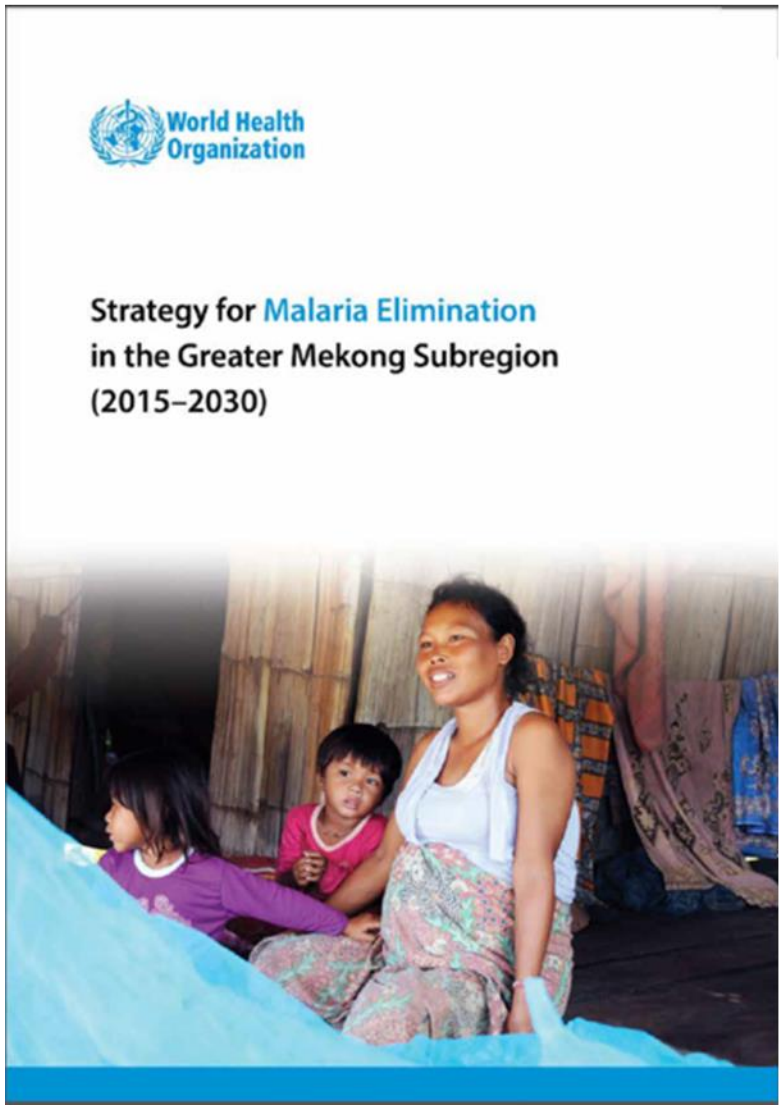


China (Yunnan) Confirmed malaria Cases (2012-June 2017



World Health Organization

GMS Strategy overview



Goals

- To eliminate malaria by **2030 in GMS**
  - eliminate *P. falciparum* malaria by **2025 (**considering the urgency of multidrug resistance)
- To maintain malaria-free status and prevent reintroduction (where transmission has been interrupted)

Objectives

1. Interrupt transmission of *P. falciparum* in areas of multidrug resistance by **2020**, and in all areas of the GMS by **2025**.
2. Reduce malaria burden in high-transmission areas (<1 /1000 pop) and initiate elimination by **2020**
3. Prevent malaria reintroduction where interrupted.

World Health Organization

GMS Strategy overview - Prioritization

- Regional level priorities
  - Interrupt transmission in areas with multidrug resistance in the border (Cambodia and Thailand);
    Reduce burden in high transmission areas (Myanmar)
    Control malaria in areas of resurgence.

- Country level priorities
  - Eliminate malaria in areas of multidrug resistance;
    Reducing burden in areas of transmission;
    Local analysis and better targeting of measures to high risk groups

World Health Organization

GMS Strategy 2015-2030: milestones and targets

Malaria elimination policies/ NSPs developed/updated

Low transmission:
- surveillance for elimination

High transmission:
- Universal coverage
- systems strengthening (case & ento. surv.)

- Elimination of P.f. malaria in all GMS countries;
- Malaria eliminated in Cambodia and Thailand

2015 → 2016 → 2017 → 2020 → 2025 → 2030

Malaria transmission interrupted in 60% of districts in Thailand

- P.f. transmission interrupted in all areas of MDR
- P.f malaria eliminated in Cambodia;
- Malaria eliminated in China including Yunnan;
- All 1st level subnational areas in GMS in elimination mode

Malaria eliminated in all GMS

World Health Organization

GMS Strategy overview - Key interventions

Case detection and management

- Universal access to quality diagnosis (public, private sector and community)
- Treatment with ACTs, primaquine for both *P. falciparum* (single dose) and *P. vivax* (anti-relapse therapy)
- Management of severe cases and imported cases to prevent deaths

Disease prevention in transmission areas

- Vector control
- Drug based approaches

Malaria case and entomological surveillance

- Mandatory notification
- Case based malaria surveillance
- Case, foci investigation and response
- Entomological surveillance
- Outbreak detection and response
- Vigilance

Supporting elements

- Innovation and research
- Enabling environment, including HSS, multi sector engagement and governance

World Health Organization

MME team (Based in WHO Cambodia): key areas for work

Partnership forum

- Information sharing on partners' activities
- Communication between partners and WHO (e.g. new guidelines)
- Facilitation of discussions over specific policies/activities

Advocacy and external communication

- High level advocacy (e.g. political forums, WHA)
- External Communication
- Resource mobilization

Support cross-country initiatives (as needed)

- Data sharing platform
- Cross-boarder collaboration
- Assessment of innovation (e.g. vaccine, new vector control)

World Health Organization

Discussion on the WHO Support to GMS

- The launch of the malaria elimination strategy in the GMS leads to a shift from the Emergency Response to Artemisinin Resistance (ERAR) to a Mekong Malaria Elimination programme (MME).

- WHO will focus on support at country level

- Numerous partners are present in the subregion, many funded by donors providing earmarked funding towards malaria elimination in the GMS.

- WHO will strengthen the work with partners at country, regional and global levels

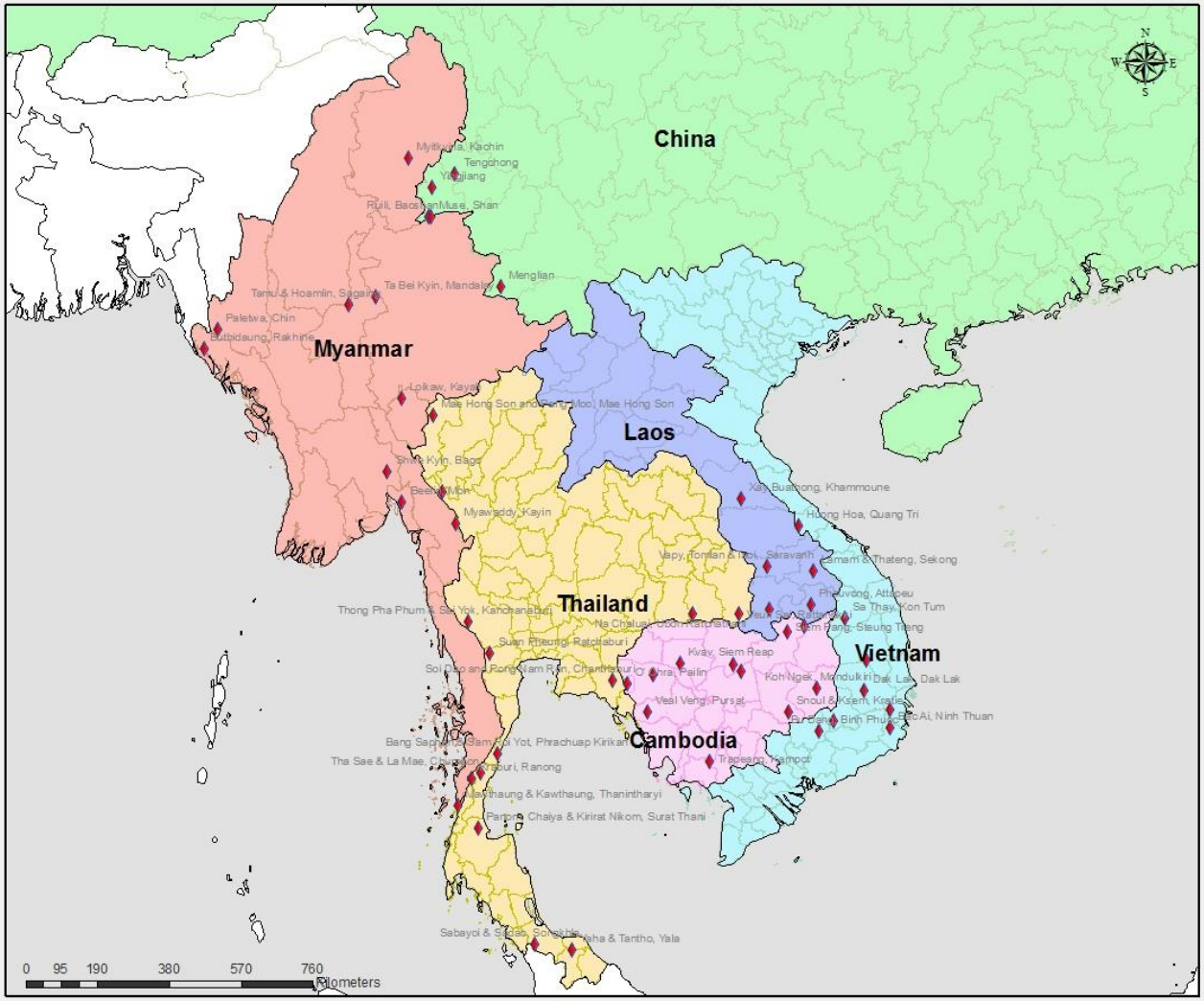
World Health Organization

Regional coordination: tracking progress, surveillance

- Data elements and indicators agreed
  - Burden reduction and elimination phases
  - Case based pilot project implemented in Moung Russay, Cambodia
  - Emphasis on improving quality of data, completeness of data, timely submissions, inclusion of data from community provides, volunteers, partners and private sector.
- Regional Data Sharing Platform → Mekong Malaria Elimination Database
  - Collation and sharing of data among all countries
  - Sharing of malaria data in border provinces
  - Regular production of Bulletins and reports
  - Mapping
- Country level:
  - Malaria elimination database and Case-based surveillance
    - Cambodia – starting in one out of 18 OD
    - Myanmar- 52/284 township (MS Access, others- Excel → DHIS2)
    - Lao PDR – in 3/18 provinces in Northern Lao
- Data managers recruited in countries
  - Cam, Lao, MMR

World Health Organization

TES sites in the six Greater Mekong Subregion (GMS) countries, 2013 to 2016



51 sentinel sites

Cambodia - 11
Lao PDR - 6
Myanmar - 11
Thailand - 13
Viet Nam - 6
Yunnan, China - 4

Legend

- TES sentinel sites

GMS countries

- Cambodia
- China
- Lao People's Democratic Republic
- Myanmar
- Thailand
- Viet Nam

Source: WHO

Map Production:
Malaria Unit,
Department of communicable diseases
WHO/SEARO
05/07/2017

World Health Organization
Regional Office for South-East Asia

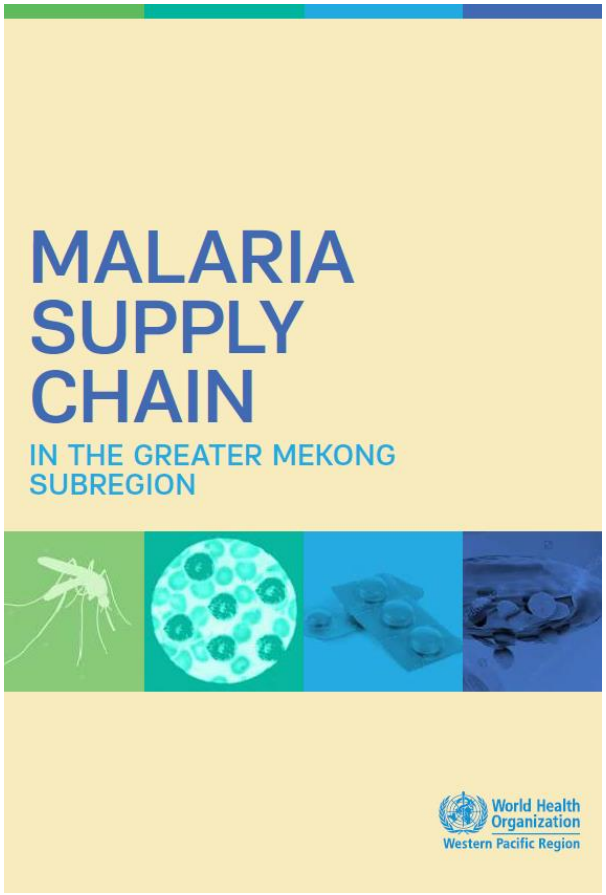
Regional Pharma Grant

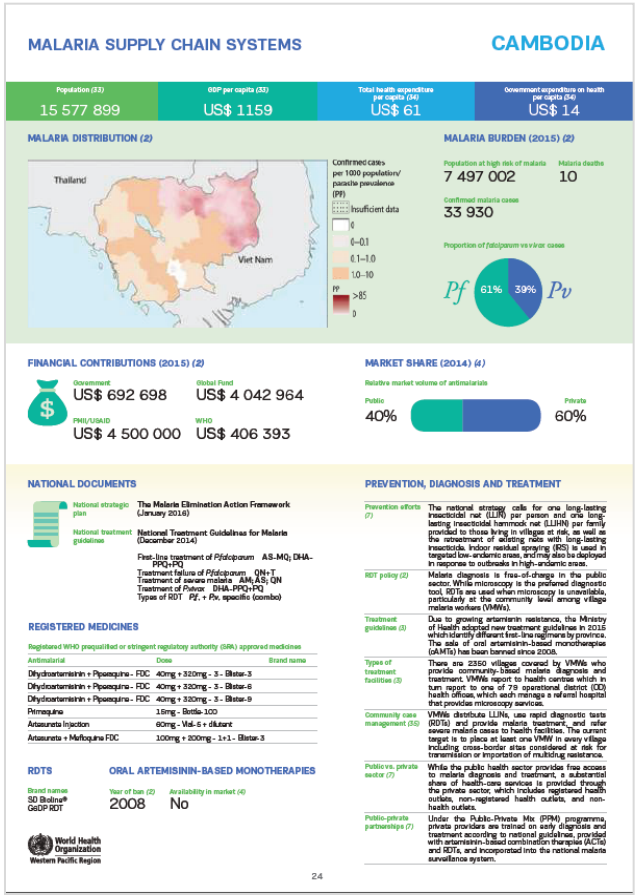
Main activities:

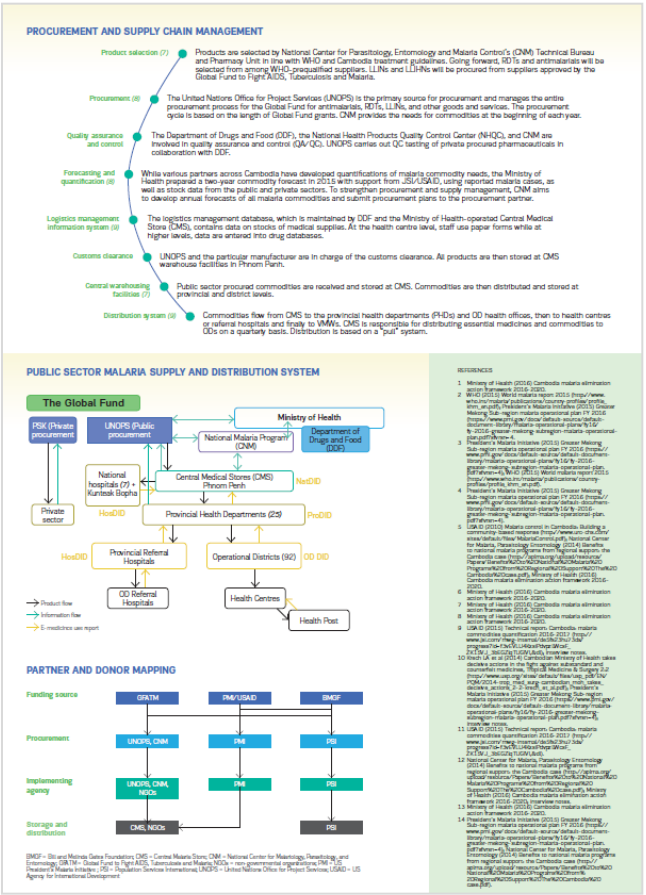
1. Assessment of Malaria Supply Chain in Greater Mekong Sub-region
2. Greater Mekong Sub-regional Workshop on Regulatory Actions to counter Substandard and Falsified Medicines, 25-27 April 2017, Bangkok, Thailand
3. Rational Drug Use Survey in Cambodia and Lao PDR
4. Surveillance on Quality, Source, and Prevalence of Antimalarial Medicines in the GMS

World Health Organization

1. Assessment of malaria supply chain in the GMS







Full report at
http://www.wpro.who.int/essential_medicines/documents/9789290618294-malaria-supply-chain-in-GMS/en/

World Health Organization

2. Substandard and falsified medicines

GMS Workshop on Regulatory Actions to counter Substandard and Falsified Medicines, 25-27 April 2017, Bangkok, Thailand

- 3 day workshop co-organized by WHO and ADB
- 1 day with senior officials to discuss cross-border collaboration and to identify key potential regulatory actions needed to eliminate poor-quality medicines
- 2 day hands-on training on post-market surveillance, inspection, and reporting to WHO global surveillance

Recommendations for Member States

- strengthen post-marketing surveillance to monitor the quality and safety of products in the market;
- strengthen the regulatory capacity at the provincial level and to promote coordination and collaboration with other enforcement agencies such as police and customs;
- share information with neighbouring countries in the GMS on substandard and falsified products detected and any products withdrawn or recalled from the market; and
- promote reporting to the WHO Rapid Alert System to help and alert other Member States.

Recommendations for WHO:

- to reflect the suggestions from Member States into Global Fund Regional Artemisinin-resistance Initiative (RAI-2) malaria pharmaceutical activities to support Member States in strengthening regulatory capacity;
- to support Member States to organize bilateral cross-border workshops in provinces along country borders to strengthen regulatory capacity, collaboration and information sharing; and
- to strengthen the capacity of Member States in regulatory enforcement actions on manufacturers, wholesalers, distributors and retailers to tackle poor-quality products.

World Health Organization

3. Rational drug use survey in Cambodia and Lao PDR

Facility-based fever management survey in public and private sectors in Lao PDR and public sector in Cambodia

- Update:
  - Lao PDR data collection completed.
  - Substantial delay in Cambodia and study restarted in October 2017
- Challenges
  - Delays in getting national ethical approval
  - Low fever patients due to seasonal variations
  - Extension of data collection days resulted in extra contracts
  - Challenging communication with CNM and provincial program managers

World Health Organization

3. Rational drug use survey in Cambodia and Lao PDR

Health Care provider interviews in Lao PDR, March 2017

First training session, 27th -28th Feb 2017, Phnom Penh, Cambodia

World Health Organization

4. Surveillance on quality, source, and prevalence of antimalarial medicines in the GMS

- Countries covered: Lao PDR, Myanmar, Thailand, Viet Nam (unable to get clearance in Cambodia)
- Products collected: total of 386 samples in 4 countries
- Challenges
  - Government clearance and national ethical approval
  - Environmental risks (flooding in Vietnam) and logistical issues

Major findings from the survey

Total samples tested: 386

16 different antimalarials tested

1. Artemether inj
2. AL tab
3. Artesunate inj
4. Artesunate tab
5. Chloroquine inj
6. Chloroquine tab
7. Hydroxychloroquine tab
8. DHA-PIP tab
9. Doxycycline tab
10. Mefloquine tab
11. Primaquine tab
12. Pyrimethamine tab
13. Quinine inj
14. Quinine tab
15. Quinine capsultes
16. Sulfamethoxazole-pyrimethamine tab

Country	Total samples tested
Lao PDR	65
Myanmar	83
Thailand	122
Vietnam	116
Total	386

oAMT were found in Myanmar, Thailand, and Vietnam

Products are mainly from

- Guilin Pharma in China
- Mediplantex from Vietnam
- Atlantic Lab in Thailand

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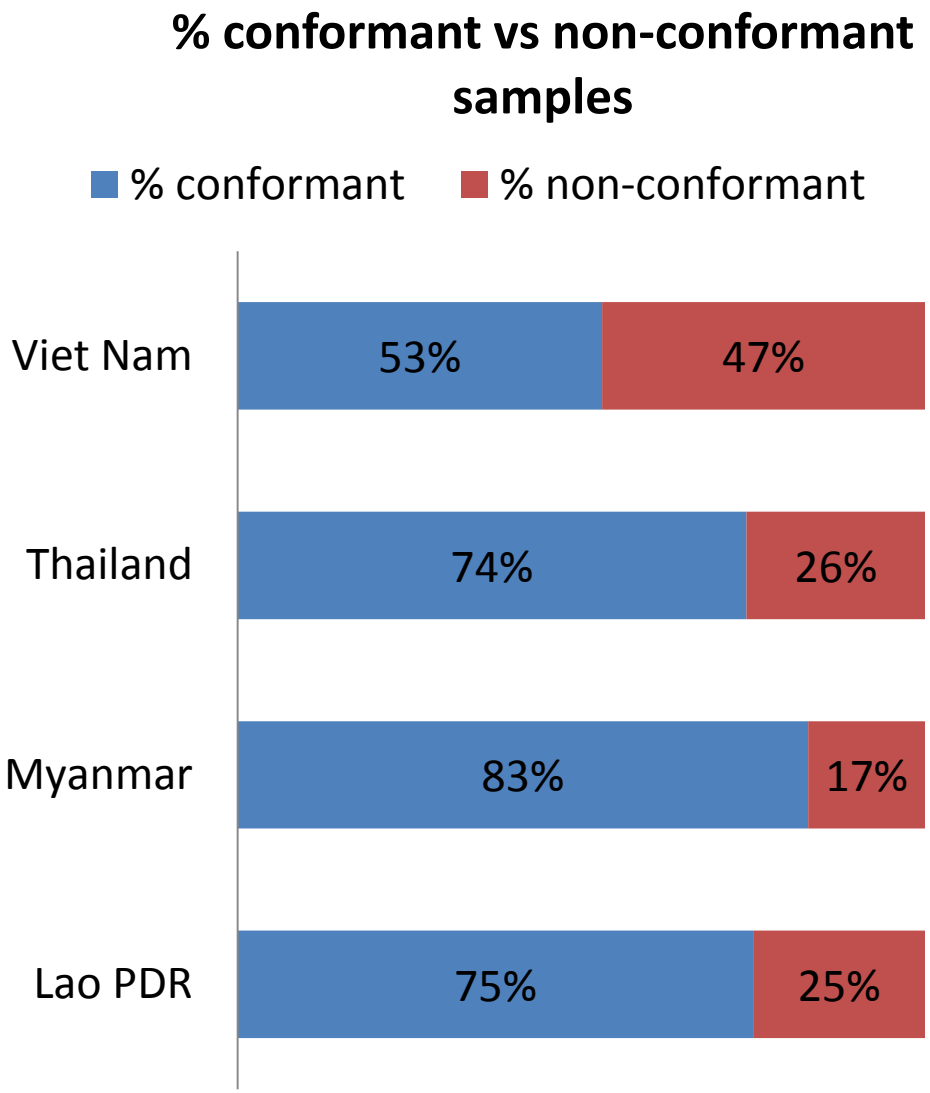


- All products passed ID test i.e. API is present
- 17% did not have the right amount of API
- 4% failed dissolution test
- 21% failed impurity test
- Non-conformant products had been found with similar frequency in public and private sector
- No substantial difference between registered and non-registered products in terms of failure rate

World Health Organization

Regional coordination

Domain	Status
Capacity building & technical collaboration	• GMS elimination training (WHOCC JIPD, China) • Elimination Operation Framework (March 2017) • National trainings in Cambodia, Lao, Myanmar, Thailand.
Cross border collaboration	• Cross-border initiatives: Lao-Thailand, Cambodia-Thailand, China-Myanmar, Myanmar-India/Bangladesh • MMP strategy and toolkit developed, in country MMP pilots
Product quality	• Assessment of Malaria Supply Chain in Greater Mekong Sub-region • Rational Drug Use Survey in Cambodia and Lao PDR • Surveillance on Quality, Source, and Prevalence of Antimalarial Medicines in the GMS
Priority research	• Priority Operational research areas discussed at RSC, Hanoi • Support of several ongoing research projects
Surveillance, M& E	• Surveillance Strengthening meeting for WPR completed June 2017 • Regional data sharing platform (DHIS2)-pilot completed in Cambodia, starting in Lao • Intense TES monitoring through networks (GMS and beyond)
Coordination and governance	• Leading and supporting NSP implementation • Malaria Programme Review completed in Vietnam Sept 2017 • Facilitate regional and partner coordination (annual forum) • Advocacy & communication (website, newsletter)

World Health Organization

Country progress reports

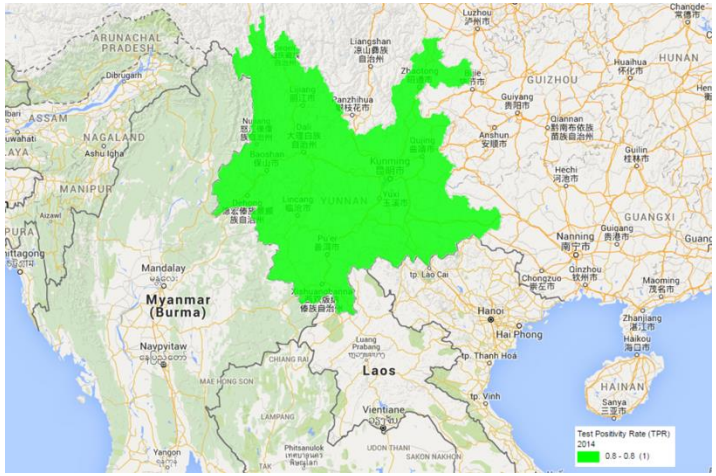
Country updates: **Cambodia**

- NSP: MEAF 2016 – 2020 launched
- AS-MQ introduced as first line treatment
- Delay in implementation of low dose Primaquine
- Case based pilot of surveillance completed
- Continued delays in roll out of DHIS2 based surveillance (CHAI-BMGF)
- Reported increase of malaria incidence in 6 provinces since June 2017 – currently being investigated. A reported decline in malaria in 16 provinces.
- TES:
  - AS-MQ 100% effectiveness (TES April 2017) but >20% D3 (+) in Pursat, Siem Reap, Kratie and Kg Speu
  - DHA-PPQ: >60% failure in Siem Reap and Kampong Speu; 30-40% failures in Oddar Meanchey, Steung Treng and Battambang
- External Competency Assessment of Microscopists completed June 2017
- Issues relating to payments for village malaria workers resolved in August 2017

World Health Organization

Country updates: **China**

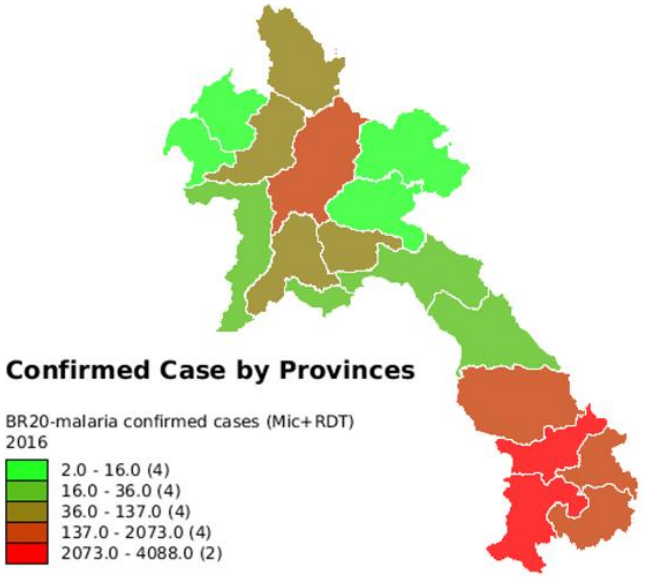
- No indigenous cases reported since October 2016
- Preparations on going for subnational verification
- Elimination training in JIPD
- Third Cross border collaboration meeting between Myanmar and China completed in September 2017
- External Competency Assessment of Microscopists completed Sept 2017



World Health Organization

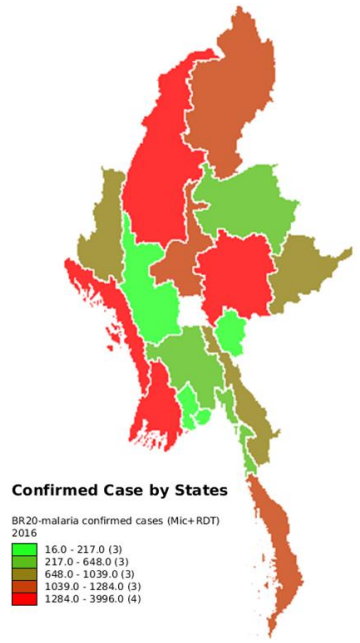
Country updates: **Lao People's Democratic Republic**

- NSP 2016 – 2020 launched;
- Malaria stratification completed.
- Integration of malaria data into DHIS2 completed.
- Case based surveillance to be piloted in three Northern provinces
- National slide bank established
- Low dose Primaquine for falciparum not fully implemented
- Microscopy refresher trainings completed for Northern and southern provinces
- MMFO training completed for programme managers of Central Lao
- TES studies ongoing and more provinces now reporting >10% failures to AL; preliminary results of DHA-PIP as alternative ACT in Champasak province not very promising



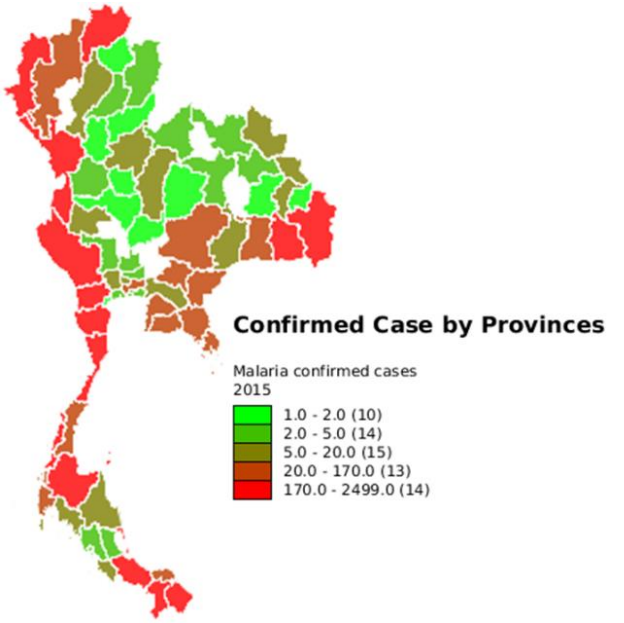
Country updates: **Myanmar**

- Launching of the national Malaria Strategic Plan, M&E plan and Malaria Elimination Plan
- Malaria Elimination training for Central and State/Regional Malaria Control Programme Staff completed in May 2017
- National Training on Management of Malaria Field operations for mid-level programme managers completed June 2017
- Following documents drafted/launched;
  - National Malaria QA/QC Manual 2017
  - Integrated Community Malaria Volunteer guideline 2017
  - Guidelines and SOPs for entomological monitoring and surveillance 2017
  - Malaria Surveillance in Elimination Settings – an operational manual 2017
  - National Malaria Reference Laboratory Strategic Plan 2017-2021
  - National Malaria Microscopy Standard Operating Procedures 2017
- Third China-Myanmar Cross Border Meetings held Sept 2017
- TES results- continued good response to ACTs



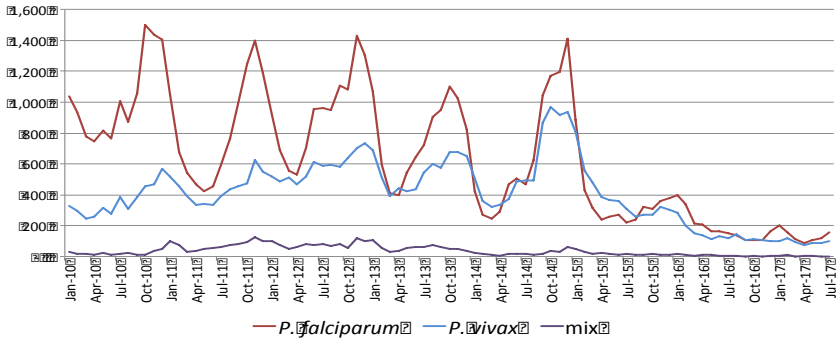
Country updates: **Thailand**

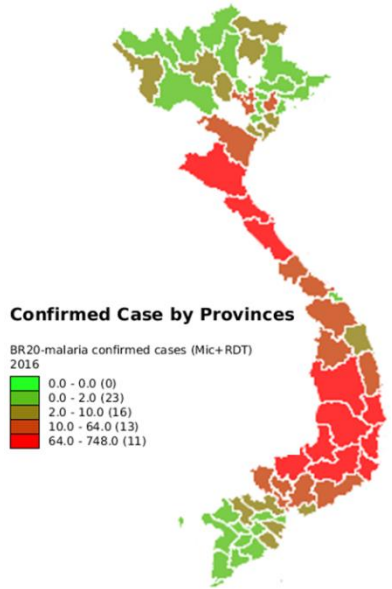
- **Policy:** NSP for malaria elimination (2017-2026), launched, 25 April 2016
- **Treatment:** Drug policy changed in Q2/2015 to DHA-PIP, rollout in 2016
- **Diagnostics:** establishing a national malaria reference laboratory
- **Surveillance:** near real time case based, foci registry established.
- **Human resource:** gradual shift from vertical to general health system
- **Capacity:** Cascading trainings for Malaria Elimination
- **Innovation:** piloting iDES (integrated drug efficacy surveillance)



Country updates: **Viet Nam**

- Cases reduced significantly
  - 4,161 confirmed cases in 2016
- Costed NSP aims for elimination by 2030
- Significant gaps in universal access to diagnosis and treatment among most at risk populations
- TES: more provinces with increasing failures to DHA-PIP confirmed by K13 and Plasmepsin2-3 mutations
- ECA of microscopists done August 2017
- Expanding resistance to ACT (PPQ) - AS+MQ introduced in in Binh Phuoc and Dak Nong Province





Policy and implementation challenges

- Country ownership
- Gaps in achieving universal access to services and commodities
- Complex partners landscape requiring better coordination of efforts for impact
- Continuing evolving of drug resistance to ACTs
- Delays in rollout of policies and guidance (Updating of NTGs and Primaquine)
- Lack of understanding of elimination concept and slow progress in re-orientation, change mind-set and HR reorganizations
- Weaknesses of health systems (HR limitations, HIS, health financing, PSM)

THANK YOU

World Health Organization

Technical Expert Group on Drug Efficacy and Response

1–2 June 2017

Room M 605, Headquarters, World Health Organization, Geneva, Switzerland

World Health Organization

WHO/HTM/GMP/MPAC/201712

Contents

Acknowledgments

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Abbreviations

ACT	artemisinin-based combination therapy
AL	artemether-lumefantrine
AP	atovaquone-proguanil
AS-AQ	artesunate-amodiaquine
AS-MQ	artesunate-mefloquine
AS-PY	artesunate-pyronaridine
CYP2D6	cytochrome 2D6 activity
CYP2C19	cytochrome 2C19 activity
DER	Drug Efficacy and Response
DHA-PIP	dihydroartemisinin-piperaquine
GMP	Global Malaria Programme
GPARC	Global Plan for Artemisinin Resistance Containment
GTS	Global Technical Strategy for Malaria 2016–2030
G6PD	glucose-6-phosphate dehydrogenase
GMS	Greater Mekong Subregion
$IC_{50 \text{ or } 90}$	inhibitory concentration 50% or 90%
PCR	polymerase chain reaction
Pfcrt	*P. falciparum* chloroquine resistance transporter
Pfcytb	*P. falciparum* cytochrome b
Pfdhfr	*P. falciparum* dihydrofolate reductase
Pfdhps	*P. falciparum* dihydropteroate synthase
Pfkelch 13	*P. falciparum* kelch propeller domain on chromosome 13
Pfmdr1	*P. falciparum* multidrug resistance protein 1
PSA	piperaquine survival assay
RSA	ring-stage survival assay
SMC	seasonal malaria chemoprevention
SNP	single nucleotide polymorphism
TEG	Technical Expert Group
TES	therapeutic efficacy study

Summary and recommendations

Piperaquine resistance

There is sufficient evidence to confirm *Pfplasmepsin 2-3* increased copy number as a marker of piperaquine resistance in Asia. *Pfplasmepsin 2-3* increased copy number should be incorporated into surveillance and monitoring activities globally where piperaquine is being used or considered for use. Although other mutations/amplifications may be involved in piperaquine resistance, including novel *Pfcrt* mutations, these require further research and validation before recommendations can be made.

Based on the proportion of clinical treatment failures determined in a therapeutic efficacy study (TES), no threshold for the prevalence of *Pfplasmepsin 2-3* increased copy number was recommended for treatment policy change. Nonetheless, the predictive value of *Pfplasmepsin 2-3* increased copy number prevalence with respect to clinical failure could be useful in informing the threshold at which a TES should be conducted. *Pfkelch 13* prevalence and a growing prevalence of *Pfplasmepsin 2-3* increased copy number should be considered in situations where a TES might not be feasible.

Piperaquine survival assay should be the standard in vitro assessment for piperaquine phenotype. However, IC_{90} obtained from conventional in vitro drug sensitivity assays also represents a valid method.

Markers of reinfection and recrudescence for *P. falciparum*

With regard to the current guidance on *P. falciparum* genotyping in clinical trials:

- The use of capillary electrophoresis for *msp1*, *msp1*, and *glurp* assessment should be promoted.
- Both molecular markers *msp1* and *msp2* should be genotyped for all samples. If *msp1* and *msp2* yield congruent results, this result should be reported as the overall result of the genotyping. In the few cases where there is a discrepancy between the outcomes of markers *msp1* and *msp2*, a third marker should be genotyped. This marker could be *glurp* or another validated highly diverse gene. Microsatellite markers can also be an alternative. This marker will then automatically support one of the two previous results; the majority result will then be reported as the overall result for PCR correction.
- PCR of different allelic families of *msp1* and *msp2* should be performed in different tubes in order to avoid template competition.

In terms of assessing new techniques for distinguishing recrudescence from reinfection, it was agreed that data from barcoding and amplicon sequencing could be incorporated into the planned modelling studies, along with the current length polymorphism approach. This would allow for further evaluation of the relative merits of each laboratory technique. WHO will provide data from clinical studies. New algorithms for interpreting data will be compared for their best fit to simulated data. The Technical Expert Group (TEG) recommends that once the new analysis has been completed, the guidance on *P. falciparum* genotyping should be reviewed and revised if necessary.

P. vivax molecular markers

There are no markers that can be used to differentiate between recrudescence, relapse, and reinfection, which makes it difficult to interpret primaquine efficacy and blood stage resistance studies.

There are no molecular markers of *P. vivax* resistance to chloroquine, mefloquine, or primaquine. Only markers of pyrimethamine and sulfadoxine resistance have been validated, although that treatment is not recommended for acute vivax malaria under almost all circumstances. Clinical trials of therapies for acute vivax malaria with robust therapeutic response phenotyping protocols are needed in order to inform the search for much-needed validated molecular markers of resistance.

Low/intermediate CYP2D6 activity has predictive value for recurrent *P. vivax* infections treated with effective blood schizontocides and primaquine. CYP2D6 genotyping should be included in primaquine clinical trials. The prevalence of impaired CYP2D6 varies widely among ethnic groups and may be quite common, e.g., in Southeast Asian populations the impaired *10 allele may occur in as much as 40–60% of the population. Surveys of the frequencies of impaired CYP2D6 alleles in populations exposed to risk of vivax malaria are needed.

Monitoring the efficacy of seasonal malaria chemoprevention (SMC)

An update on the TEG's previous recommendations regarding SMC is presented below.

Recommendation	Status
The ratio of malaria cases in children under 5 years versus children over 10 years	Yes
The occurrence of clinical malaria relative to the time of the previous SMC dose	Yes
The incidence of severe malaria at sentinel sites	Yes
Case–control sampling before each dose for microscopy	Case–control studies recruit clinical cases as they report to health facilities
Gametocytes	Possible, but not done
PCR relative to the time of previous SMC dose	Planned
Molecular markers of sulfadoxine-pyrimethamine and amodiaquine resistance pre-SMC	Yes, except for Senegal
Molecular markers of sulfadoxine-pyrimethamine and amodiaquine resistance post-SMC	Planned
Local capacity-building for the monitoring of molecular markers	Analyses are done in London using high throughput facility. Researchers from each country have been trained in the laboratory methods, and the project is building capacity for the design and monitoring of programmes, analysis, and interpretation
Standard membrane feeding assay	Yes
Complexity of infection from studies of parasite	Possible, but not done

genetics	
Changes in parasite diversity	Possible, but not done
Drug policy effects: the impact of SMC on first-line ACT diversity	AL is the first-line in areas where SMC is implemented

Data on the effect of SMC on molecular markers of resistance are not yet available, although baseline data indicate that parasites resistant to either sulfadoxine-pyrimethamine or amodiaquine exist at low frequencies in asymptomatic individuals. Data from Mali suggest that the risk of developing drug resistance is higher with sulfadoxine-pyrimethamine than with amodiaquine.

Strategy for antimalarial drug resistance management

The TEG agreed that it would be valuable to have a new strategy for antimalarial drug resistance management, and this should be developed and made available as soon as possible. The scope and components of the strategy presented were considered appropriate and should include the following:

- Scenario-planning, for instance in case of outbreaks of falciparum malaria in areas with multidrug resistance;
- Guidance on *P. vivax* resistance;
- New information and approaches since the GPARC;
- Distinct scenario-planning for different resistance situations;
- Consideration of all interventions using antimalarial drugs, their potential impact on resistance development, and actions that might mitigate this risk;
- Measures for containment across borders;
- Guidance on the management of suspected and confirmed treatment failures, including diagnostics and alternative treatments that can be used in remote or resource-poor areas.

An ideal format would include a generic section building on what is in the GPARC and what has been learned more recently, plus scenarios that can change over time as new evidence and tools become available.

Update on antimalarial drug efficacy and drug resistance

The TEG recommends that all putative *Pfkelch13* mutants conferring artemisinin resistance be independently verified as being associated with resistance both in genetic studies and in the RSA, ideally before publication claiming such association.

Planned activities (TES and survey) to investigate *Pfkelch13* C580Y in South America are sufficient. However, whole genome sequencing may be useful to examine backbone mutations. Ideally, resistant parasites should be collected for culture adaption.

The presence of multicopy *Pfplasmepsin 2-3* in Africa is a potential concern in terms of the use of DHA-PIP. However, additional information is required regarding the in vivo and ex vivo piperaquine-resistant phenotype in African parasites. Additional African data are needed to assess the

relationship between DHA-PIP treatment failures and molecular markers (*Pfkelch13*, *Pfplasmepsin 2-3*, and *Pfcrt*).

Triple therapies

Although TRAC 2 data are preliminary, the data support the testing of triple therapies as a potential strategy against multidrug-resistant *P. falciparum*. In particular, artemether-lumefantrine+ amodiaquine should be tested in Cambodia and Viet Nam.

Given the concern over QTc interval prolongation interval and the issues regarding the measurement of changes in QTc as malaria symptoms resolve, further analysis of QTc using alternative methods was requested.

An alternative treatment option for multidrug-resistant *P. falciparum* is to use two sequential artemisinin-based combination therapies (ACTs). This approach should be tested in clinical trials.

Atovaquone-proguanil

In the GMS, there may be a role for AP in combination with an ACT. AS-MQ+AP and AS-PY+AP are two options for testing.

Further studies are required to validate mutations as a clinically relevant molecular marker of atovaquone resistance. There may be other mutations contributing to resistance besides the *Pfcytb* mutation at position 268.

Until there is stronger evidence that a *P. falciparum Pfcytb* Y268C/N/S mutant is not transmissible, it cannot be concluded that atovaquone resistance is not transmissible.

1 Welcome and introduction of guest speakers

The list of participants is provided in Annex 1. All Technical Expert Group (TEG) members attended the meeting. Welcome to new members David Fidock, Daouda Ndiaye, and Neena Valecha, and to the invited speakers Ingrid Felger, Paul Milligan, and Mariusz Wajnarski. The Bill & Melinda Gates Foundation, Department for International Development (DFID), and Medicines for Malaria Venture were invited as observers, and representatives attended the meeting. The Global Fund to Fight AIDS, Tuberculosis and Malaria and the United States Agency for International Development were invited as observers, but were unable to attend. The meeting agenda is provided in Annex 2.

Thanks were expressed on behalf of WHO to all TEG members past and present and to the sponsors, DFID.

The role of this TEG is to advise the Drug Efficacy and Response (DER) Unit at the Global Malaria Programme (GMP) on policy and recommendations regarding drug efficacy and response. Questions directed at the TEG from the DER Unit are listed in Annex 3.

2 Declarations of interest

TEG members participating in the meeting submitted declarations of interest, which were assessed by the DER Unit at GMP.

3 Minutes and action points of TEG 2015

The minutes of the 2015 TEG were accepted and can be found at:

http://www.who.int/malaria/mpac/mpac-mar2016-teg-der-report-session3.pdf?ua=1

4 Session 1. Molecular markers: genotyping and monitoring drug resistance

4.1 Molecular markers of piperaquine resistance

Presentations

Pfplasmepsin 2-3 copy number

In 2010, dihydroartemisinin-piperaquine (DHA-PIP) was adopted as the national first-line antimalarial therapy in Cambodia. However, *P. falciparum* piperaquine resistance spread rapidly and parasites resistant to both artemisinin and piperaquine began circulating. This resulted in high DHA-PIP treatment failure rates, leading to a shift in treatment policy in these areas to artesunate-mefloquine (AS-MQ) from 2014. Although mutations in the *Pfkelch 13* propeller region have been validated as molecular markers of artemisinin resistance, there is still no molecular marker of piperaquine resistance.

The piperaquine survival assay (PSA) was developed to address the limitations of the classical isotopic in vitro assay in characterizing piperaquine-resistant isolates. This assay is able to define a reliable in vitro phenotype and has been validated retrospectively and prospectively. In studies, all isolates collected from patients presenting a recrudescence within 42 days of follow-up had PSA survival rates ≥ 10%.

Based on findings from the PSA, next-generation sequencing was performed on eight piperaquine-sensitive and 24 piperaquine-resistant strains, all with the same *Pfkelch 13* C580Y allele. Strong signals of gene amplification were detected in two genes encoding hemoglobin-digesting proteases: *Pfplasmepsin 2* and *Pfplasmepsin 3*

Note that the *Pfmdr1* gene was amplified in 5/8 piperaquine-sensitive lines, but in 0/23 piperaquine-resistant lines. Recent epidemiological studies in Cambodia have shown that most piperaquine-resistant isolates characterized by *Pfplasmepsin 2-3* gene amplification have a single *Pfmdr1* copy, while a small proportion (1%) have both *Pfplasmepsin 2-3* and *Pfmdr1* multiple copies.

Subsequent experiments demonstrated that the amplification of these genes leads to *Pfplasmepsin 2-3* mRNA overexpression, with at least 2-fold more protein in piperaquine-resistant parasites than in piperaquine-sensitive ones. In Cambodian isolates, a strong correlation was observed between *Pfplasmepsin 2-3* gene amplification (by qPCR) and PSA, with an increased *Pfplasmepsin 2-3* copy number predicting PSA survival rates ≥ 10% with a sensitivity of 97% and specificity of 98%. Clinical data from 725 patients showed that the cumulative incidence of DHA-PIP treatment failure increased with the *Pfplasmepsin 2-3* copy number, with a hazard ratio for failure of 32.2 for single versus two copies, 49.0 for single versus ≥ three copies, and 1.5 for two versus ≥ three copies. Multivariate analysis indicated that *Pfplasmepsin 2-3* copy number (single versus multiple) was the most important determinant of DHA-PIP treatment failure (adjusted hazard ratio 20.4).

A review of 405 samples collected in Pailin and 324 in Rattanakiri between 2002 and 2015 showed an increase in *Pfplasmepsin 2-3* copy number over time, mirroring the increase in treatment failures observed over this period. Notably, in Pailin, about 2% of parasites exhibited increased *Pfplasmepsin 2-3* copy number in 2002, suggesting either that this increase may be a natural polymorphism or that piperaquine-resistant parasites emerged after the massive use of DHA-PIP in the area between 2001 and 2003. Overall, data from areas of Cambodia where the *Pfkelch 13* mutation has a prevalence of > 50% indicate that the clinical efficacy of DHA-PIP at day 42 fell below 90% when the proportion of multicopy *Pfplasmepsin 2-3* parasites rose to 22% ($p < 0.0001$). Notably, multicopy *Pfplasmepsin 2-3* has no longer been detected in *Pfkelch 13* wild type parasites, contrary to data reported 5-10 years ago.

In conclusion, these findings indicate a strong association between multicopy *Pfplasmepsin 2-3* with in vitro resistance and DHA-PIP clinical failure rates. These findings have been confirmed in another set of studies conducted by a different group using another dataset and alternative approaches. In addition, multicopy *Pfplasmepsin 2-3* parasites have been reported in Viet Nam and Thailand, clustering in areas where both artemisinin resistance and piperaquine resistance are common. There is also a biological rationale for *Pfplasmepsin* 2-3 to be associated with resistance, as piperaquine affects hemoglobin degradation and heme detoxification processes.

To fully validate *Pfplasmepsin 2-3* copy number as a molecular marker of piperaquine resistance, genome edited *P. falciparum* with single/multicopy *Pfplasmepsin 2-3* would be an invaluable tool, even though editing *Pfplasmepsin 2-3* copy number is technically challenging. However, progress has been made in developing stable strains with knock down of *Pfplasmepsin 2-3* from a multicopy parasite.

Pfcrt

Four mutations in *Pfcrt* have been identified in piperaquine-resistant parasites. All of these, namely H97Y, F145I, M343L, and G353V, are exclusively on the Dd2 background and are only found in Cambodia. They have all been detected at low abundance. Genome-wide association studies with *Pfcrt* F145I found an association between these mutations and DHA-PIP treatment failure, after adjusting for the presence of amplified *Pfplasmepsin 2-3*. Introduction of the F145I mutation into Dd2 parasites also conferred piperaquine resistance in vitro and impacted the potency of multiple other antimalarial medicines. Attempts to introduce F145I into the Cambodian artemisinin-resistant background Cam3.II (harbouring the *Pfkelch 13* C580Y mutation associated with artemisinin resistance) have been unsuccessful, perhaps because of the reduced transfection efficiency observed with this strain whose cell cycle is longer than the conventional 48 hours.

Gene editing experiments on the Dd2 background showed that an experimental *Pfcrt* C101F mutation conferred piperaquine resistance and sensitized *P. falciparum* to chloroquine, quinine, and amodiaquine but not to mefloquine or lumefantrine.

The introduction of M343L and G353V into Dd2 parasites produced a piperaquine-resistant phenotype, as measured using the PSA. Dd2 parasites expressing the *Pfcrt* G353V variant had an unusually large and translucent digestive vacuole. The removal of the *Pfcrt* M343L or H97Y mutations from Cambodian piperaquine-resistant isolates restored sensitivity to piperaquine, as measured using the PSA. These mutations were also found to affect sensitivity to chloroquine, quinine, and amodiaquine, depending on the variant.

Discussion

PSA should be used if possible for in vitro phenotyping. It is difficult to get the kill curve to 0 in the isotopic assay, even when varying piperaquine doses and durations, and parasitaemia. However, the area under the inhibitory concentration curve correlates well with PSA and can provide a more dynamic range.

There are two formats for the PSA: in vitro for laboratory samples (generally done after culture adaption and synchronization) and ex vivo for fresh samples. It is not known whether these two formats provide different results. With the ring-stage survival assay (RSA) used for assessing artemisinin resistance, for example, survival is higher in the ex vivo format.

The data supporting *Pfplasmepsin 2-3* copy number, as a molecular marker of piperaquine resistance in the Greater Mekong Subregion (GMS) appear to be very strong. However, these data have been found almost exclusively in association with *Pfkelch 13* mutations, and there is no evidence that *Pfplasmepsin 2-3* copy number is a molecular marker of piperaquine resistance outside the GMS. Although treatment policy decisions should be based on clinical data from therapeutic efficacy studies (TESs), it may be possible to define a threshold for the prevalence of *Pfplasmepsin 2-3* multicopy number at which a TES should be triggered.

Although rare, the existence of parasites with multiple copies of both *Pfplasmepsin 2-3* and *Pfmdr1* is worrying. In many areas of Cambodia, the prevalence of multicopy *Pfplasmepsin 2-3* parasites remains high. DHA-PIP has been removed only recently, so it may be too early to observe a decrease in the prevalence of multicopy *Pfplasmepsin 2-3* parasites in areas where DHA-PIP is no longer used. The propensity to lose multiple copy numbers in the absence of selective pressure might depend on

the parasite's genetic background. The dynamics of *Pfplasmepsin 2-3* de-amplification in the absence of drug pressure has not been established; for other *P. falciparum* genes, the de-amplification or silencing of copies tends to be quite a rapid process, as is the opposite process of re-expressing or re-amplifying once selective pressure is restored.

As with mefloquine in Cambodia and Thailand, in China, where piperaquine was used as a monotherapy, piperaquine resistance emerged in the absence of artemisinin resistance. It is not yet known whether piperaquine resistance can emerge in the absence of artemisinin resistance where DHA-PIP is used, for example in Africa. Since areas of low transmission and high DHA-PIP use are most vulnerable to the development of piperaquine resistance, monitoring in these areas of Africa would be valuable.

In Cambodia, patterns of *Pfcrt* mutation may be very regional. It is possible that the *Pfcrt* mutations listed above have been missed because there has been no analysis of the individual variants of Dd2 and examination of how they map individually to the PSA. The genome-wide association studies should be re-analysed and compared, taking into account the Dd2 variants.

Evidence suggests that parasites with *Pfcrt* mutation are often less fit (potentially due to high levels of accumulated globin-derived peptides). Fitness might be restored in such parasites harbouring multiple copies of *Pfplasmepsin 2-3* (where hemoglobin processing might be accelerated). It is possible that these additional mutations drive high-level piperaquine resistance.

The *Pfcrt* C350R mutation, identified by the Institut Pasteur de Cayenne in South America, has been shown to reverse chloroquine resistance, but in vitro has induced a significant decrease in piperaquine susceptibility. There is no evidence that this mutation is present in Asian parasite strains.

4.2 New evidence on *msp1*, *msp2*, and *glurp* as markers of reinfection and recrudescence

Presentation

In 2007, WHO published guidance on genotyping to identify parasite populations for clinical trials on antimalarial efficacy (Methods and techniques for clinical trials on antimalarial drug efficacy: genotyping to identify parasite populations. Geneva: World Health Organization, 2008). Recommendations were to compare *P. falciparum* parasite genotypes sequentially in pre- (day 0) and post-treatment samples (day X of treatment failure) using *msp1*, *msp2*, and *glurp* as markers of new infection vs. recrudescence. Given the experience gained by different groups and advances in laboratory techniques over the last 10 years, a reappraisal of the 2007 guidance may be required.

Issues that have been identified include poor quality of PCR execution and analysis (especially with respect to reading the agarose gels), PCR bias towards short fragments, template competition, and limitations in the use of the sequential decision algorithm for deciding on recrudescence or reinfection, particularly in high transmission areas where multiplicity of infections is high and many coinfection clones compete with each other during PCR amplification.

A plan for examining the validity of revised algorithms that differentiate between *P. falciparum* recrudescence and reinfection was presented, along with a comparison of different methods for

generating and interpreting results, and modelling studies and statistical methods to support a genotyping strategy that best fits the simulated data.

4.3 Reinfection/recrudescence: pros and cons of other methods (microsatellites, barcoding, and amplicon sequencing)

Presentation

There are a number of different markers and techniques that can be used to differentiate individual parasite infections. Depending on the research question, however, all have their advantages and limitations.

Single nucleotide polymorphism (SNP)-based genotyping leverages independent, neutral alleles with a high frequency of variation within or between populations. This approach may be useful in low transmission settings with evidence of single-clone infections; it may be more difficult to use in areas of higher transmission dominated by polygenomic infections, or in areas of very low transmission with highly inbred parasite populations in which gene variation is greatly reduced.

Microsatellites have the same limitations as *msp1*, *msp2*, and *glurp*.

Amplicon sequencing requires that a reinfection exhibit a distinct amplicon haplotype that is not represented in the initially treated infection. The technique has greater discriminatory power if two amplicons are used rather than one. There is the possibility to multiplex PCRs of many samples and several molecular markers in order to investigate recrudescence versus reinfection and drug-resistance markers simultaneously. Since the PCR products are of a similar size, there is also less size–length polymorphism bias than with *msp1*, *msp2*, and *glurp* genotyping. Although this approach is likely to be highly cost–efficient, it is currently less deployable in most field settings, since the approach relies on deep sequencing strategies.

4.4 Barcoding to genotype *Plasmodium* in TES: experience in South-East Asia

Presentation

A 10-SNP barcode has been validated using DNA from reference strains. The barcode was applied to clinical data from Cambodian clinical trials, using *msp1, msp2*, and *glurp* genotyping in parallel, with good concordance between the two techniques. The results from the barcoding were easier to interpret in the low transmission area characterized by limited parasite diversity; this would not be the case, however, with polygenomic infections. Furthermore, in very low transmission settings with limited parasite types, it may be challenging to distinguish reinfection by the same parasite type. Compared to PCR gels, the 10-SNP barcode requires more sophisticated equipment and is more expensive.

Global discussion of the three presentations

Many laboratories are able to obtain consistent PCR results. However, high transmission settings present greater challenges, given the relatively higher levels of polygenomic infections. There should be continued efforts to build capacity and improve training and quality control for PCR in local laboratories. Blood samples should be sent to a centralized laboratory for quality control in order to improve quality.

Capillary electrophoresis was recommended in the 2007 WHO document. In many cases, this procedure can be outsourced to a site that has such facilities available. For example, in surveillance studies, PCR can be performed locally, with subsequent capillary electrophoresis conducted at a separate site. Local investigators can download the electronic capillary electrophoresis results as one file per sample for analysis, which also serves as a quality control method for the PCR.

Re-examination of the genotyping guidelines is warranted, and the plan to validate new approaches seems reasonable. Data from various sources will be used, comparing results obtained through the current length polymorphism approach (using *msp1*, *msp2*, and *glurp*) and, if possible, amplicon sequencing and barcoding. A pharmacokinetic/pharmacodynamic model will be used to simulate recrudescence and reinfection following drug treatment. The performance of the different methodologies and algorithms will be assessed in terms of their ability to distinguish between new infection and recrudescence in settings of different transmission intensity. Note that the model can incorporate the genetic structure of the local population, so background allele frequency estimates will be needed. Different laboratory techniques may be more appropriate given the setting and transmission level. The following points should be considered when developing new guidelines:

- In very low transmission settings, where only a few *P. falciparum* strains are circulating, it may be difficult to use molecular markers to distinguish recrudescence from reinfection, since reinfection can be with the same parasite strain or a closely related one. In these settings, methods with a higher resolution, such as microsatellite typing or genetic barcoding, are likely to perform better than conventional *msp1*, *msp2*, and *glurp* genotyping; however, these methods will also fail to distinguish recrudescence from reinfection when the reinfecting genotype is the same.
- The cost and complexity of each technique and whether it can be performed locally or centrally should also be considered. Quality control should always be centralized and should start from blood samples, not from extracted DNA.
- For any new laboratory procedures, there should be guidance on the workflow of samples and if/when it might be appropriate to centralize them. Local ownership of data and analysis should be ensured, even if the laboratory procedures are carried out elsewhere.
- The interpretation of molecular markers may be aided by understanding the background diversity of these markers at the study site. For routine surveillance in very low transmission settings, it might be appropriate to assume that a recurrent infection is a recrudescence without having to genotype the infecting strains; for research and TES studies that have potential impact on drug policies, however, the best available genotyping method should be used. These methods require proper validation and should be reproducible by other laboratories.
- Techniques and decision-making should be simple and clear-cut.

4.5 Update on *P. vivax* molecular markers

Presentation

The hypnozoite dictates *P. vivax* malaria epidemiology, diagnosis, treatment, prevention, control, and elimination. However, it is not yet possible to detect hypnozoites, and there is little

understanding of the potential size of the hypnozoite reservoir. Available studies suggest that over 80% of acute attacks derive from relapses (Thai and Papua New Guinea cohort studies). Relapse latency varies between 17 days and 5 years, and the risk, timing, and number of relapses varies both geographically and temporally. Relapse latency is a key consideration when assessing the effectiveness of anti-hypnozoite drugs, as a long latency period and short follow-up will fail to detect relapses. Studies indicate that treatment failures occur mostly within 6 months and more rarely within 8 months with rapid-relapsing tropical Asian strains. Temperate strains almost certainly require at least 8 months follow-up and ideally 12 months.

The radical cure of *P. vivax* requires blood schizontocidal therapy plus primaquine. *P. vivax* resistance to chloroquine has been confirmed in many sites throughout the world, although data are relatively sparse. A recurrence before day 17 is likely to be recrudescence; between days 17 and 28, it could be recrudescence, relapse, or reinfection. Chloroquine resistance is defined as any recurrence with chloroquine+desethylchloroquine whole blood concentrations > 100 ng/ml at the time of recurrence. Primaquine treatment before day 28 creates a bias in the in vivo test, as primaquine also has blood schizonticidal activity. There is no validated genetic marker of chloroquine resistance in *P. vivax*.

There is no information on parasite resistance to primaquine, as recrudescence, relapse, and reinfection cannot be differentiated genetically. Determining whether the recurrence is homologous or heterologous is irrelevant for assessing primaquine anti-hypnozoite efficacy. Consequently, there is no primaquine-resistant phenotype for *P. vivax*, either clinically or in animal models, and molecular markers of *P. vivax* primaquine resistance cannot currently be validated.

There is some evidence that there are two distinct populations of sporozoites, destined to either progress through the life cycle (tachysporozoites) or enter dormancy as hypnozoites (bradysporozoites). The ratio of these populations appears to be strain-specific. It is not yet known whether the fate of each population is a genetic or epigenetic trait; if genetic, there might be the possibility of using a genetic marker to distinguish relapse from reinfection or recrudescence.

In order to assess primaquine efficacy, high-dose primaquine must be used (30 mg x 14 days), with supervised adherence and no exposure to reinfection. Follow-up must be at least 4 months, preferably 12 months. Ideally, a rapidly eliminated schizonticide medicine should be used (quinine or artesunate). Moreover, failure to relapse may be caused by a lack of hypnozoites. Although such studies are challenging to conduct, available data from infections acquired in Indonesian Papua indicate that primaquine anti-hypnozoite efficacy is about 85% after 1 year of follow-up.

However, primaquine resistance may not be the cause of the 15% treatment failure rate. Importantly, primaquine is a prodrug, and its efficacy apparently depends on cytochrome 2D6 activity (CYP2D6) for conversion to the active metabolites. Poor/intermediate CYP2D6 metabolizers are at significantly greater risk of relapse compared to normal metabolizers. Thus, there appears to be a population of patients who will be naturally less responsive to primaquine. As such, primaquine resistance can only be concluded if therapeutic levels of the active metabolites are achieved. As currently those metabolites cannot be readily measured, CYP2D6 genotyping provides important clues in the form of predicted metabolic activity levels using the AS-Model scoring system.

Discussion

Trials of anti-hypnozoite therapy must include a relapse control arm with only effective blood schizontocidal activity in order to identify the natural relapse behaviour of local strains. In areas of chloroquine resistance, chloroquine alone should not be used, as chloroquine-resistant *P. vivax* could confound the results.

Chloroquine+primaquine will have greater blood schizontocidal efficacy than chloroquine alone. Therefore, assessing chloroquine efficacy without primaquine therapy is essential for identifying chloroquine resistance as a clinical and public health problem. Although chloroquine+primaquine is almost universally recommended, in practice, the inclusion of primaquine carries the risk of intravascular hemolysis due to glucose-6-phosphate dehydrogenase (G6PD) deficiency; consequently, primaquine is often not applied.

It may be important to understand that patients in endemic areas may experience relapse infections due to hypnozoites from one or more prior infections. In other words, it is possible to have relapses at any time from hypnozoites, so it cannot be assumed that failure before day 17 is a recrudescence and chloroquine failure. Nonetheless, any persistent or rapidly reappearing parasitaemia (< 17 days) that is overcoming relatively high levels of chloroquine in the blood is certainly resistant to the drug, regardless of the parasite source (i.e., from hypnozoites or recrudescent infections).

The evidence for tachysporozoite and bradysporozoite phenotypes comes from human infection studies conducted in the 1940s/50s. In the studies, a North Korean late relapse strain with no primary infection was thought to have a dominant population of bradysporozoites, unlike the rapidly relapsing tropical Chesson strain that had a dominance of tachysporozoites. In addition, Thai strains distinguished by circumsporozoite markers consistently produced fixed proportions of hypnozoites versus tissue schizonts. There are various explanations for these findings, including potential interactions with the hepatocyte and genetic/epigenetic differences. It is not clear whether the predisposition exists at the sporozoite stage (genetically programmed) or after entering the hepatocyte (epigenetic commitment phenomena). Gametocyte production is epigenetically regulated and commits a consistent proportion of sporozoites to sexual differentiation; this may also be the case for hypnozoites.

At present, strains with the potential for primaquine resistance are not under significant selection pressure, as primaquine is likely not being applied at sufficient levels to sustain the selection pressure to drive resistance. Chloroquine resistance has been identified at many sites, including the Indonesian archipelago and malarious Western Pacific, where high rates of resistance have necessitated the adoption of an artemisinin-based combination therapy (ACT) as first-line *P. vivax* therapy. Elsewhere, however, chloroquine remains an affordable and convenient treatment option and should not be abandoned without firm evidence that treatment failure is occurring at rates exceeding 10% of acute attacks. However, in the future, a single treatment protocol for *P. falciparum* and *P. vivax* may be appropriate, including a presumptive radical cure with primaquine where these two species are sympatric (i.e., almost everywhere). Such a practice would require great improvements in the safety of primaquine hypnozoitocidal therapy, e.g., with robust G6PD deficiency screening or close clinical monitoring. Primaquine is too dangerous a drug to apply broadly without the necessary measures in place to prevent serious harm, including possible loss of life.

4.6 Recommendations: Session 1

Piperaquine resistance

There is sufficient evidence to confirm *Pfplasmepsin 2-3* increased copy number as a marker of piperaquine resistance in Asia. *Pfplasmepsin 2-3* increased copy number should be incorporated into surveillance and monitoring activities globally where piperaquine is being used or considered for use. Although other mutations/amplifications may be involved in piperaquine resistance, including novel *Pfcrt* mutations, these require further research and validation before recommendations can be made.

Based on the proportion of clinical treatment failures determined in a TES, no threshold for the prevalence of *Pfplasmepsin 2-3* increased copy number was recommended for treatment policy change. Nonetheless, the predictive value of *Pfplasmepsin 2-3* increased copy number prevalence with respect to clinical failure could be useful in informing the threshold at which a TES should be conducted. *Pfkelch 13* prevalence and a growing prevalence of *Pfplasmepsin 2-3* increased copy number should be considered in situations where a TES might not be feasible.

PSA should be the standard in vitro assessment for piperaquine phenotype. However, IC_{90} obtained from conventional in vitro drug sensitivity assays also represents a valid method.

Markers of reinfection and recrudescence for *P. falciparum*

With regard to the current guidance on *P. falciparum* genotyping in clinical trials:

- The use of capillary electrophoresis for *msp1*, *msp1*, and *glurp* assessment should be promoted.
- Both molecular markers *msp1* and *msp2* should be genotyped for all samples. If *msp1* and *msp2* yield congruent results, this result should be reported as the overall result of the genotyping. In the few cases where there is a discrepancy between the outcomes of markers *msp1* and *msp2*, a third marker should be genotyped. This marker could be *glurp* or another validated highly diverse gene. Microsatellite markers can also be an alternative. This marker will then automatically support one of the two previous results; the majority result will then be reported as the overall result for PCR correction.
- PCR of different allelic families of *msp1* and *msp2* should be performed in different tubes in order to avoid template competition.

In terms of assessing new techniques for distinguishing recrudescence from reinfection, it was agreed that data from barcoding and amplicon sequencing could be incorporated into the planned modelling studies, along with the current length polymorphism approach. This would allow for further evaluation of the relative merits of each laboratory technique. WHO will provide data from clinical studies. New algorithms for interpreting data will be compared for their best fit to simulated data. The TEG recommends that once the new analysis has been completed, the guidance on *P. falciparum* genotyping should be reviewed and revised if necessary.

P. vivax molecular markers

There are no markers that can be used to differentiate between recrudescence, relapse, and reinfection, which makes it difficult to interpret primaquine efficacy and blood stage resistance studies.

There are no molecular markers of *P. vivax* resistance to chloroquine, mefloquine, or primaquine. Only markers of pyrimethamine and sulfadoxine resistance have been validated, although that treatment is not recommended for acute vivax malaria under almost all circumstances. Clinical trials of therapies for acute vivax malaria with robust therapeutic response phenotyping protocols are needed in order to inform the search for much-needed validated molecular markers of resistance.

Low/intermediate CYP2D6 activity has predictive value for recurrent *P. vivax* infections treated with effective blood schizontocides and primaquine. CYP2D6 genotyping should be included in primaquine clinical trials. The prevalence of impaired CYP2D6 varies widely among ethnic groups and may be quite common, e.g., in Southeast Asian populations the impaired *10 allele may occur in as much as 40–60% of the population. Surveys of the frequencies of impaired CYP2D6 alleles in populations exposed to risk of vivax malaria are needed.

5 Session 2. Monitoring the prophylactic effect of preventive treatment

5.1 Monitoring efficacy of seasonal malaria chemoprevention in the ACCESS-SMC project

Presentation

Seasonal malaria chemoprevention (SMC) was scaled up in 2015 and 2016, having been recommended by WHO in 2012. A total of about 26 million monthly treatments with sulfadoxine-pyrimethamine plus amodiaquine were administered to about 6.7 million children in 2015, and about 60 million treatments (15 million children) were administered in 2016. In 2016, 12 countries (Burkina Faso, Cameroon, Chad, Gambia, Ghana, Guinea, Guinea-Bissau, Mali, Niger, Nigeria, Senegal, and Togo) had SMC programmes. Funding has been secured to maintain similar coverage in 2017. In 2015 and 2016, about half of the treatments were delivered through the UNITAID-funded ACCESS-SMC project (3.8 million children in 2015 with 14.5 million treatments) and 8 million in 2016 with 30 million treatments) in seven countries (Burkina Faso, Chad, Gambia, Guinea, Mali, Niger, and Nigeria). Children < 5 years have been included, except in Senegal and parts of Mali where treatment is for children under 10. In parts of northern Mali, SMC is provided for all ages.

Nearly 100 million SMC treatments have been delivered since 2012. SMC has been deployed most extensively in Mali (33 million treatments), Burkina Faso (15 million), Niger (12.5 million), and Nigeria (12.2 million). In most countries, in ACCESS-SMC implementation areas between 2015 and 2016, more than 80% of children have received SMC. Overall coverage with at least three monthly treatment cycles in ACCESS-SMC areas has reached 73%.

The London School of Tropical Medicine and Hygiene is working with research groups in Burkina Faso, Chad, Gambia, Guinea, Mali, Niger, Nigeria, and Senegal to measure SMC coverage and quality of delivery, assess the impact of SMC on malaria, support pharmacovigilance, monitor the efficacy of

SMC drugs, and evaluate the prevalence of markers of sulphadoxine-pyrimethamine and amodiaquine resistance. The baseline data on molecular markers of resistance in Burkina Faso, Chad, Gambia, Guinea, Mali, Niger, and Nigeria were presented.

The impact of SMC can be seen in comparison to control districts with no SMC, for example a 45% reduction in cases in children under 5 in Burkina Faso. The impact can also be seen with a reduction in malaria cases in children under 5 versus children over 10. Malaria mortality in children under 5 has also been reduced relative to pre-SMC data and relative to children over 10. In Senegal, a reduction in the number of malaria deaths in hospital in children under 10 was observed following introduction of SMC for that age group in the southern regions of the country.

In 2015, baseline community surveys to monitor drug-resistance markers were conducted in areas that were yet to start SMC (with the exception of Gambia, which started SMC in 2014) in children under 5 and those aged 10–30 years. A total of 2000 samples were collected in each group in each area, with a total target sample size of 28 000. Markers were *Pfcrt* (CVMNK, CVIET, and SVMNT), *Pfmdr1* (86, 184, and 1246), *Pfdhfr* (51, 59, and 108), and *Pfdhps* (431, 436, 437, 540, 581, and 613). Of the 21 024 samples tested, 3448 (16.4%) were *P. falciparum* positive and 2324 have been genotyped so far.

The prevalence of *Pfcrt* and *Pfmdr1* markers reflects the drug combinations most used for first-line malaria treatment in recent years. *Pfcrt* CVIET was most prevalent in Chad (54%) and Gambia (53%), and at very low prevalence in Nigeria (5%).

Amodiaquine resistance was defined as *Pfcrt* CVIET plus *Pfmdr1* 86Y+184Y. *Pfmdr1* YY haplotypes were uncommon, seen in only five samples (one in Burkina Faso, four in Niger). One of the samples from Niger had *Pfcrt* CVIET and *Pfmdr1* YY.

Sulfadoxine-pyrimethamine resistance was defined as *Pfdhfr* 51+59+108 plus *Pfdhps* 437+540. Eight samples (0.33%) – seven from Guinea and one from Niger – carried *Pfdhfr* triple and *Pfdhps* double mutations. None of these samples carried *Pfmdr1* YY. Molecular marker data from Senegal were not presented.

In conclusion, mutations associated with sulfadoxine-pyrimethamine- and amodiaquine-resistant phenotypes were at low prevalence in the study areas before the roll-out of the SMC intervention. Further analyses will examine samples from malaria cases in relation to the period of time since SMC. Surveys will be repeated after the 2017 transmission season with complete results in 2018. There is a need for a longer term plan to monitor resistance.

Discussion

The data represent an excellent baseline set, and the TEG would be grateful to see post-SMC data when they become available. The only trends observed so far have been from Mali, where molecular markers of sulfadoxine-pyrimethamine resistance increased after SMC. For example, the *Pfdhfr-Pfdhps* quintuple mutant genotype increased from 1.6% to 7.1% ($p = 0.02$). However, the prevalence of *Pfmdr1* 86Y decreased from 26.7% to 15.3% ($p = 0.04$), with no change for *Pfcrt* K76T.

The presence of *Pfdhfr-Pfdhps* quintuple mutants, even at low prevalence, is a concern, as it might reflect other selection pressures on sulfadoxine-pyrimethamine, for example from intermittent preventive treatment during pregnancy; in this case, looking for markers in pregnant women would be valuable. Note that artemether-lumefantrine (AL) is being used as first-line malaria treatment in

the SMC countries and thus may be deterring the development of amodiaquine resistance. Given that artesunate-amodiaquine (AS-AQ) is difficult to buy in the SMC areas, there is probably little use of amodiaquine other than for SMC. However, there is nothing to deter the development of resistance to sulfadoxine-pyrimethamine.

Increased prevalence of resistance markers alone will not trigger policy change; this will depend on SMC efficacy determined through case–control studies.

It is not clear whether the prevalence of resistance markers will be the same in asymptomatic cases as in malaria patients. However, there are some surveys being conducted in patients presenting to health centres, and rapid diagnostic test cassettes have been retained from health facilities near survey sites. Resistance markers from these sources should be compared with those from asymptomatic infections. Although drugs for SMC are being used for their preventive effect, they must also have efficacy to clear parasites from asymptomatic individuals.

Membrane-feeding studies to assess impact on transmission are being performed in Burkina Faso, but results are not yet available. With only children under 5 included in SMC, there will be no effect on transmission. However, where children up to 10 are included, transmission may be reduced. In many areas with SMC programmes, there is a substantial burden of severe disease and malaria mortality in older children. Accordingly, there is a rationale for extending SMC to older children in these areas. At present, however, the goal of SMC is to reduce clinical cases and infant mortality.

5.2 Recommendations: Session 2

Monitoring the efficacy of SMC

An update on the TEG's previous recommendations regarding SMC is presented below.

Recommendation	Status
The ratio of malaria cases in children under 5 years versus children over 10 years	Yes
The occurrence of clinical malaria relative to the time of the previous SMC dose	Yes
The incidence of severe malaria at sentinel sites	Yes
Case–control sampling before each dose for microscopy	Case–control studies recruit clinical cases as they report to health facilities
Gametocytes	Possible, but not done
PCR relative to the time of previous SMC dose	Planned
Molecular markers of sulfadoxine-pyrimethamine and amodiaquine resistance pre-SMC	Yes, except for Senegal
Molecular markers of sulfadoxine-pyrimethamine and amodiaquine resistance post-SMC	Planned
Local capacity-building for the monitoring of molecular markers	Analyses are done in London using high throughput facility. Researchers from each country have been trained in the laboratory methods, and the project is building capacity

	for the design and monitoring of programmes, analysis, and interpretation
Standard membrane feeding assay	Yes
Complexity of infection from studies of parasite genetics	Possible, but not done
Changes in parasite diversity	Possible, but not done
Drug policy effects: the impact of SMC on first-line ACT diversity	AL is the first-line in areas where SMC is implemented

Data on the effect of SMC on molecular markers of resistance are not yet available, although baseline data indicate that parasites resistant to either sulfadoxine-pyrimethamine or amodiaquine exist at low frequencies in asymptomatic individuals. Data from Mali suggest that the risk of developing drug resistance is higher with sulfadoxine-pyrimethamine than with amodiaquine.

6 Session 3. Prevention and treatment of multidrug-resistant malaria

6.1 Outline of a strategy for antimalarial drug resistance management

Presentation

The purpose of the presentation was to discuss the potential development of a new strategy for antimalarial drug resistance management.

Recent WHO guidance on how to manage and respond to antimalarial drug resistance has mainly focused on the threat of artemisinin resistance. This includes guidance given in the *Global plan for artemisinin resistance containment* (GPARC), released in 2011, and the *Emergency response to artemisinin resistance in the Greater Mekong Subregion, regional framework for action 2013–2015,* released in 2013.

The *Global technical strategy for Malaria 2016–2030* (GTS), adopted at the World Health Assembly in 2015, highlights the potential of antimalarial drug resistance to seriously weaken the effectiveness of malaria responses and to erode the gains achieved. Therefore, the GTS recommends the monitoring and management of antimalarial drug resistance.

Since the development of the GPARC, the understanding of artemisinin resistance has improved, and the growing impact of resistance to ACT partner drugs is being recognized. Furthermore, while the GPARC focused on the risk of spread of resistance, resistance to antimalarial medicines can emerge independently in different locations. In December 2016, a WHO Expert Review Group concluded that there is "a significant risk of artemisinin and partner drug resistance outside the GMS – either via spontaneous emergence or importation, and spread". In part because of these developments, countries have been requesting concrete guidance on the management of antimalarial drug resistance.

The proposed new strategy for antimalarial resistance management aims to protect the efficacy of all malaria treatments. The key target audience is Ministries of Health and partners mainly working

at the country level. The presentation outlined the suggested components of the strategy and placed added emphasis on the planning and implementation of activities at the country level.

GMP will develop the strategy with potential support from the TEG. Feedback from countries will be sought at regional and subregional meetings, with an online consultation for additional feedback from a range of stakeholders.

Discussion

The TEG agreed that a new strategy for antimalarial drug resistance management would be valuable. Scenario-planning for different eventualities was thought to be particularly useful, potentially with some regional adaptions. While there is already a strategy for malaria elimination for the GMS, additional technical recommendations focusing on problems related to antimalarial drug resistance are needed, for instance recommendations on how to detect and manage treatment failures. Planned components laid out in the new strategy should be detailed enough for programmes to verify what additional activities might be needed in order to avert or manage resistance. It would be useful if the scenario-planning component could be in a format that is easy to update.

The TEG thought the following issues should be included in the strategy:

- Scenario-planning, for instance in case of outbreaks of *P. falciparum* malaria in areas with multidrug resistance;
- *P. vivax* resistance to antimalarial drugs;
- The factors contributing to the emergence and spread of resistance;
- New information and approaches since the GPARC and the rationale for changes since the GPARC was published;
- The potential impact on the risk of resistance following changes in transmission and immunity.

In addition, while resistance may emerge independently, the spread of resistance remains a major threat. In light of this, population movements and border areas require special attention, and regional (rather than country-level) strategies may be required. The strategy should also outline the current landscape of use of antimalarial medicines, not just for treatment. All interventions using antimalarial drugs should be considered, as should the potential impact on resistance development and actions that might mitigate this risk. The risk–benefit of interventions using antimalarial drugs should be taken into account, since any use of drugs can carry a risk of resistance. However, this risk does not mean that the intervention should be discouraged. Ensuring effective quality control of drugs is a key factor in resistance management, and the strategy should include practical steps to monitor and improve quality control. Finally, guidance on the management of suspected and confirmed treatment failures should be provided, including diagnostics and alternative treatments that can be used in remote or resource-poor areas.

6.2 Update on antimalarial drug efficacy and drug resistance

Presentation

All ACTs are vulnerable to artemisinin resistance, though even in areas with a high prevalence of *Pfkelch13* mutations, ACTs retain treatment efficacy in the absence of resistance to the partner drug.

In the event of partner drug resistance, treatment failure rates increase and, unless containment measures are in place, resistance will spread. However, containment can work. For example, malaria elimination efforts at the Thai–Myanmar border have resulted in a large decrease in malaria cases, reducing the potential for spread to other regions.

In Cambodia, DHA-PIP failure rates at day 42 are < 5% for parasites that have wild type for piperaquine resistance markers with regarless presence of *Pfkelch13* wild type or *Pfkelch13* mutant (mainly C580Y). However, failure rates increase to around 20% for parasites with *Pfkelch13* wild type or multicopy *Pfplasmepsin 2-3*, and to 45% for those harbouring both markers.

In 2015–2016, a TES was conducted at five sites in Cambodia. At three of the sites, parasites were found that had both multicopy *Pfmdr1* and multicopy *Pfplasmepsin 2-3* at frequencies of 5.8% (Siem Reap), 4.2% (Pursat), and 2.0% (Kampong Speu). These frequencies are worrying as *Pfmdr1* increased copy number had virtually disappeared in Cambodia. Therefore, the appearance of the double mutant with markers of both piperaquine and mefloquine resistance is concerning. It was hypothesized earlier that the two drugs might have competing resistance mechanisms that allowed DHA-PIP to drive multicopy *Pfmdr1* out of the population and restore sensitivity to mefloquine in the region after it had been failing.

Four ACTs have already failed in Cambodia, and if AS-MQ efficacy is lost, there are limited alternative options available. Studies are underway with artesunate-pyronaridine (AS-PY) in Eastern Cambodia, but previous data showed high failure rates in Western Cambodia with subsequent failure on DHA-PIP retreatment. These failures remain unexplained, as there is no cross-resistance between piperaquine and mefloquine with pyronaridine.

In Africa, *Pfkelch13* mutations are rare and have not been associated with artemisinin resistance. In 2013, *Pfkelch13* M579I was isolated from a Chinese worker who had malaria 8 weeks after returning from Equatorial Guinea; however, the circumstances of the case and the parasite origin are not well documented.

Partner drug resistance is an emerging issue in Africa, with treatment failure rates increasing for AL. Treatment failures have also been observed in travellers. Cases are not well documented for resistance, and in some instances antimalarial drug levels may have been sub-therapeutic. However, treatment failure in travellers should be considered a red flag for reduced sensitivity. As in the GMS, AL treatment failures in Africa are not associated with *Pfmdr1* increased copy number.

Multicopy *Pfplasmepsin 2-3* was detected at a rate of 8.7% in the Comoros in 2013 and 5.3% in Mozambique in 2016.

In South America, *Pfkelch13* C580Y mutants were detected in Guyana in 2010. These mutants had a distinct microsatellite profile, indicating an origin independent of those observed in South-East Asia. While a TES in 2014 found no *Pfkelch13* mutants, a 2016 survey (n = 691) confirmed the presence of *Pfkelch13* C580Y in two neighbouring regions in the north-west of the country at a prevalence of 9.4% (9/96) in region 1 Barima-Waini, 0.6% (3/477) in region 7 Cuyuni-Mazaruni, and 1.9% (13/691) overall. The prevalence of *Pfkelch13* mutants appears to be seasonal, as they were detected between April and September, with a peak in June. TES is planned in region 7 and in the capital city, Georgetown. The survey will continue in 2017.

In conclusion, the data reaffirm the need for an urgent and continued intensive regional malaria elimination campaign in the GMS both to prevent a resurgence of malaria caused by multidrug-resistant parasites and to contain these parasites, thereby preventing their spread to other areas. Surveillance for artemisinin and partner drug resistance needs to be continued and strengthened in the GMS. Outside the GMS, surveillance is needed to detect potential de novo resistance or introduction of resistant parasites. Where surveillance signals a potential threat to leading ACTs, effective alternative ACTs should be identified and implemented before resistance reaches critical levels.

Discussion

The report of the *Pfkelch13* M579I parasite isolated from a Chinese worker who had returned from Equatorial Guinea provides insufficient information to conclude that this parasite was acquired in Africa or was of African origin, or that this mutation confers artemisinin resistance, as the RSA value is marginal. The TEG recommends that all putative *Pfkelch13* artemisinin-resistant mutants be independently verified both for genetics and in the RSA, ideally before publication. It is reassuring that, despite two surveys, no further *Pfkelch13* mutants have been detected in Equatorial Guinea. Thus, there is no evidence that an artemisinin-resistant *Pfkelch13* mutant parasite population has become established or spread in the country.

The relevance of multicopy *Pfplasmepsin 2-3* to piperaquine resistance in African strains that lack mutant *Pfkelch13* is unknown. Therefore, it should not be concluded that DHA-PIP would fail in areas where these parasites have been detected. If multicopy *Pfplasmepsin 2-3* is a natural polymorphism, as suspected, then there will be a small background prevalence of these parasites.

The presence of multicopy *Pfplasmepsin 2-3* in Africa is a potential concern in terms of the use of DHA-PIP. Drug pressure from DHA-PIP (through its use in mass drug administration and via the private sector) could further select multicopy *Pfplasmepsin 2-3*. However, additional information is required as to the in vivo and ex vivo piperaquine-resistant phenotype in African parasites. Data are needed on the relationship between DHA-PIP treatment failures and molecular markers (*Pfkelch13*, *Pfplasmepsin 2-3*, and *Pfcrt*). Such data may have already been collected in various clinical trials of DHA-PIP in Africa, but will need to be examined.

Policy change should be based on therapeutic efficacy. However, a high prevalence of multicopy *Pfplasmepsin 2-3* or a trend of increasing prevalence should trigger further investigation.

Given the diversity of *Pfkelch13* mutations in Cambodia conferring artemisinin resistance, it is interesting that *Pfkelch13* C580Y is the only *Pfkelch13* mutation that has been detected in South America. In Cambodian parasites, *Pfkelch13* C580Y is the most fit *Pfkelch13* mutation. It is possible that this is the only mutation with great enough fitness to survive in the South American setting. The findings could also be the result of a homogeneous population structure with few different circulating parasites. However, the situation in Cambodia today may not be so relevant to the South American setting, as *Pfkelch13* mutations have been evolving in South-East Asia for 15 years.

The malaria situation in Venezuela has gained recent attention, as cases have soared following the breakdown of malaria control and health care provision. The regions of Guyana in which *Pfkelch13* C580Y has been detected border Venezuela. Although microsatellites have been examined, whole genome sequencing may be useful to examine backbone mutations. Ideally, resistant parasites

should be captured for culture adaption, but this presents logistical challenges. There are also plans to obtain *P. falciparum* and *P. vivax* samples from a regional hospital in a mining area of Venezuela.

6.3 Update on TRAC 2: preliminary results of triple therapies

Presentation

The TRAC 2 study is evaluating triple therapies for uncomplicated *P. falciparum* malaria. The study aims to enroll 1800 subjects at 15 sites in eight countries, mostly in South-East Asia and India, but with one site in the Democratic Republic of Congo. Treatment comparisons are DHA-PIP versus DHA-PIP+mefloquine, AL versus AL+amodiaquine, and AS-MQ versus DHA-PIP+mefloquine, depending on the malaria treatment guidelines in the country. So far, 294 patients have been enrolled. The study will be completed in mid-2018.

Preliminary data indicate high failure rates for DHA-PIP in Cambodia, Viet Nam, and Thailand, but 100% efficacy with DHA-PIP+mefloquine. In Cambodia, the control arm was switched to AS-MQ after the start of the study, in line with the malaria treatment policy, with an efficacy rate of 100%. In Myanmar, efficacy was found to be 100% with both DHA-PIP and DHA-PIP+mefloquine. Data with AL have shown a few recurrent infections, but so far there have been no recurrent infections in the AL+amodiaquine arm in South-East Asia or India. In Democratic Republic of Congo, recurrent infections with both AL and AL+amodiaquine appear to be high, but these are likely reinfections; PCR correction has not yet been done.

Although data remain limited, all triple therapies have generally been well tolerated with no concerning laboratory parameters. No further prolongation of QTc interval has been observed with DHA-PIP+mefloquine compared to DHA-PIP without mefloquine. A slight QTc prolongation has been found with amodiaquine added to AL, although this is attributable to amodiaquine rather than to an interaction between the drugs.

Discussion

In areas where DHA-PIP is failing, the effectiveness of DHA-PIP+mefloquine treatment would rely to a large extent on the mefloquine component; as such, AL+amodiaquine may be the preferred triple therapy. Also, the initial rationale of combining DHA-PIP with mefloquine assumed that the drugs have competing resistance mechanisms. In light of the data presented at the TEG on parasites from Cambodia with both multicopy *Pfmdr1* and multicopy *Pfplasmepsin 2-3*, this rationale might need to be reassessed.

AS-AQ have limited efficacy in Cambodia. However, lumefantrine and amodiaquine have opposing resistance mechanisms, and this combination should now be tested in Cambodia and Viet Nam – countries with high DHA-PPQ failure rates.

Mefloquine has been found to increase the risk of QTc interval prolongation when associated with halofantrine and quinine. However, this does not seem to be the case for mefloquine and piperaquine. QTc prolongation with DHA-PIP+mefloquine has been evaluated in healthy volunteers and is now being assessed in patients, with reassuring preliminary results.

Multidrug-resistant *P. falciparum* threatens to undermine all current treatment options in Cambodia. If triple therapies were to be introduced into treatment protocols, the components would need to be co-packaged. For the potential introduction of triple therapies, not only must the

necessary data be obtained, but also appropriate product presentation must be developed and manufacturing and distribution logistics put in place; all aspects need to be worked on simultaneously.

Ideally, alternative approaches using currently approved drugs should be tested along with triple therapies. One option for multidrug-resistant *P. falciparum* is to use two sequential ACTs. As there are issues with neutropenia at cumulative doses of artemisinin over 24 mg/kg, further safety data are needed. Adherence may be an issue, although co-packaging will help. If there are no alternatives, however, adherence problems will have to be overcome.

6.4 Use of atovaquone-proguanil in the context of a containment project in Cambodia

Presentation

In 2011, atovaquone-proguanil (AP) became the first-line treatment in Pailin because of high treatment failure rates with DHA-PIP. Although AP's efficacy was initially 100% in 2008–2009 and 2010, a study conducted in 2012–2013 reported high AP treatment failure rates. Whereas 4/24 patients had wild type parasites at day 0 and Y268N (n = 3) or F263T (n = 1) mutants at day 28, 1/24 treatment failures had the *Pfcytb* Y268C mutation present at day 0 and day 28. The remaining 19 patients had wild type parasites at codon 268 at day 0 and day 28. A survey found *Pfcytb* mutant parasites in 13/295 samples, but it is unknown whether these were 'day 0' or recrudescence samples. A temporal relationship was found between the use of AP in Pailin and the increase in the frequency of AP treatment failures or prevalence of *Pfcytb* mutant parasites.

In Africa, it was reported that atovaquone-resistant mutations can occur spontaneously and that another mutation can be involved (I258M) in treatment failure. In addition, there is some evidence that atovaquone-resistant *P. berghei* parasites are not transmissible to mosquitoes.

6.5 Role of atovaquone-proguanil and artesunate+atovaquone-proguanil for the treatment of multidrug-resistant malaria in Cambodia

Presentation

A TES was conducted in Cambodia comparing AP and artesunate+AP in 205 volunteers with *P. falciparum* or mixed *P. falciparum/P. vivax* infection. Primaquine 15 mg was co-administered with the first dose of study medicine to all patients by direct-observed treatment. The study was conducted at two sites: Anlong Veng in the north and Kratie in the east. Treatment efficacy at day 42 was just above 90% with no difference between arms. Efficacy at Kratie was higher than at Anlong Veng. Artesunate+AP appeared to have some beneficial effects on gametocyte carriage relative to AP alone, but the effect on transmission has not been evaluated.

The ex vivo susceptibility of parasites did not differ between patients treated with AP and those treated with artesunate+AP. IC_{50} values for AP were low, suggesting that treatment failures could be caused by inadequate atovaquone exposure; however, pharmacokinetic data are pending and IC_{50}s could not be established for all recrudescent samples. Cytochrome 2C19 (CYP2C19) metabolizes proguanil into the active metabolite cycloguanil, which has limited antimalarial activity against

parasites carrying *Pfdhfr* mutations. However, there is an unknown mechanism by which proguanil potentiates the antimalarial activity of atovaquone. Thus, theoretically, slow CYP2C19 metabolizers should have higher proguanil levels and consequently higher atovaquone antimalarial activity. Conversely, rapid metabolizers should have lower proguanil levels and therefore lower atovaquone potentiation. No data were available on the CYP2C19 status of patients in this study.

Nearly all isolates at both sites carried *Pfkelch13* C580Y. *Pfcytb* Y268C was detected in only 1/14 treatment failures. Based on the amplicon deep sequencing of the blood sample with confirmed *Pfcytb* mutation at recrudescence, it appears that resistance developed during AP therapy. There may be other atovaquone-resistant *Pfcytb* mutations not detected through Sanger sequencing.

Combinations of AP and other antimalarial drugs could be investigated as a stop-gap measure against multidrug-resistant *P. falciparum*.

In conclusion, both regimens were well tolerated, but there was no clinical benefit to artesunate+AP over AP alone, probably because of extensive artemisinin resistance in the region. However, the addition of artesunate may reduce gametocyte carriage, and the potential impact on transmission requires evaluation. The probability of selecting for atovaquone-resistant mutants has been estimated as 1 in 500 treatments in other studies. The emergence of AP resistance would have important implications for chemoprophylaxis in South-East Asia.

Discussion

In published case reports, the presence of the *Pfcytb* Y268C mutation in the samples from patients with treatment failure was associated with a significant rise of atovaquone IC_{50}s (at least 1000-fold). However, this marker has not been validated and it is not known whether *Pfcytb* mutations affect resistance at the population level.

Most treatment failures with AP appear to be occurring without detectable *Pfcytb* mutations. Therefore, treatment failures may also be related to the pharmacokinetics of AP, but drug levels in the blood are needed to further investigate these failures. Even in the absence of detectable *Pfcytb* mutations, failure rates appear to be around 10%, which may potentially be related to CYP2C19.

Until there is additional evidence that *P. falciparum Pfcytb* Y268C/N/S mutants are not transmissible, it cannot be concluded that atovaquone resistance is not transmissible. However, membrane feeding assays or direct feeding studies would be needed to assess the true risk of infectivity of field parasites with *Pfcytb* mutations.

Where artemisinin is failing, combining AS with AP, which has an intrinsic failure rate of 10% (even in the absence of detectable *Pfcytb* mutation), will not work. If AP were to be redeployed, it would need to be in combination with an ACT (not doxycycline), preferably one with a partner drug with a long half-life, e.g., either AS-MQ or AS-PY. Drug–drug interactions may limit the potential combinations available. The safety of multiple combination drugs is always a concern.

Currently, the efficacy of AS-MQ is 100% in Cambodia. Although adding AP to this would not improve efficacy, it might protect the combination from developing resistance and provide safety information on the triple combination.

The efficacy of AS-PY in Western Cambodia is not as high as it is elsewhere, although the reasons for this are unclear. Ideally, AP should be partnered with a drug with high efficacy so as to minimize the

risk of resistance development. However, if it becomes the case that there are no other options in Cambodia, it might be useful to know now whether an AS-PY+AP combination has higher efficacy than AS-PY alone and to obtain additional safety data.

6.6 Recommendations: Session 3

Strategy for antimalarial drug resistance management

The TEG agreed that it would be valuable to have a new strategy for antimalarial drug resistance management, and this should be developed and made available as soon as possible. The scope and components of the strategy presented were considered appropriate and should include the following:

- Scenario-planning, for instance in case of outbreaks of falciparum malaria in areas with multidrug resistance;
- Guidance on *P. vivax* resistance;
- New information and approaches since the GPARC;
- Distinct scenario-planning for different resistance situations;
- Consideration of all interventions using antimalarial drugs, their potential impact on resistance development, and actions that might mitigate this risk;
- Measures for containment across borders;
- Guidance on the management of suspected and confirmed treatment failures, including diagnostics and alternative treatments that can be used in remote or resource-poor areas.

An ideal format would include a generic section building on what is in the GPARC and what has been learned more recently, plus scenarios that can change over time as new evidence and tools become available.

Update on antimalarial drug efficacy and drug resistance

The TEG recommends that all putative *Pfkelch13* mutants conferring artemisinin resistance be independently verified as being associated with resistance both in genetic studies and in the RSA, ideally before publication claiming such association.

Planned activities (TES and survey) to investigate *Pfkelch13* C580Y in South America are sufficient. However, whole genome sequencing may be useful to examine backbone mutations. Ideally, resistant parasites should be collected for culture adaption.

The presence of multicopy *Pfplasmepsin 2-3* in Africa is a potential concern in terms of the use of DHA-PIP. However, additional information is required regarding the in vivo and ex vivo piperaquine-resistant phenotype in African parasites. Additional African data are needed to assess the relationship between DHA-PIP treatment failures and molecular markers (*Pfkelch13*, *Pfplasmepsin 2-3*, and *Pfcrt*).

Triple therapies

Although TRAC 2 data are preliminary, the data support the testing of triple therapies as a potential strategy against multidrug-resistant *P. falciparum*. In particular, AL+amodiaquine should be tested in Cambodia and Viet Nam.

Given the concern over QTc interval prolongation interval and the issues regarding the measurement of changes in QTc as malaria symptoms resolve, further analysis of QTc using alternative methods was requested.

An alternative treatment option for multidrug-resistant *P. falciparum* is to use two sequential ACTs. This approach should be tested in clinical trials.

Atovaquone-proguanil

In the GMS, there may be a role for AP in combination with an ACT. AS-MQ+AP and AS-PY+AP are two options for testing.

Further studies are required to validate mutations as a clinically relevant molecular marker of atovaquone resistance. There may be other mutations contributing to resistance besides the *Pfcytb* mutation at position 268.

Until there is stronger evidence that a *P. falciparum Pfcytb* Y268C/N/S mutant is not transmissible, it cannot be concluded that atovaquone resistance is not transmissible.

Annex 1: List of participants

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Annex 2: Meeting agenda

Thursday 1 June 2017		
09:00–09:20	Welcome **P. Alonso – Director GMP** **A. Dondorp – Chair TEG DER**	
09:20–09:30	Declaration of interest **P. Ringwald**	
09:30–09:50	Minutes and action points of the last TEG meeting **A. Dondorp**	→ For information
Session 1: Molecular markers: genotyping and monitoring drug resistance		**Purpose of session and expected outcomes**
09:50–11:00	i) Molecular markers of piperaquine resistance **D. Ménard 20'** **D. Fidock 5'** **C. Plowe 5'** **Discussion 40'**	→ For information and decision
11:00–11:30	Coffee/tea break	
11:30–12:40	ii) New evidence on *mps1, msp2*, and *glurp* as markers of reinfection and recrudescence **I. Felger 30'** iii) Reinfection/recrudescence: pros and cons of other methods (microsatellites, barcoding, and amplicon sequencing) **S. Volkman 30'** iv) Barcoding to genotype *Plasmodium* in TES: experience in South-East Asia **D. Ménard 10'**	→ For information and decision

12:40–14:00	**Lunch**	
14:00–15:00	**Discussion 60'**	
15:00–16:00	v) Update on *P. vivax* molecular markers: - genotyping to differentiate homologous and heterologous infections; - molecular markers of drug resistance. **K. Baird 30' + 30'**	→ For information and decision
16:00–16:30	Coffee/tea break	
Session 2: Monitoring the prophylactic effect of preventive treatment		**Purpose of session and expected outcomes**
16.30–17:20	Monitoring efficacy of seasonal malaria chemoprevention in the ACCESS-SMC project **P. Milligan 25' + 25'**	→ For information
Session 3: Prevention and treatment of multidrug resisitant malaria		**Purpose of session and expected outcomes**
17:20–18:00	Outline of a strategy for antimalarial drug resistance management **C. Rasmussen 20' + 20'**	→ For information and comments
Friday 2 June 2017		
Session 3: Prevention and treatment of multidrug-resistant malaria		**Purpose of session and expected outcomes**
8:30–9:00	Update on antimalarial drug efficacy and drug resistance **P. Ringwald 30'**	→ For information
9:00–09:30	Update on TRAC 2: preliminary results of triple therapies **A. Dondorp 30'**	→ For information
9:30–10:30	**Discussion 60'**	

10:30–11.00	**Coffee/tea break**	
11:00–11:15	Use of atovaquone-proguanil in the context of a containment project in Cambodia **P. Ringwald 15'**	→ For information and comments
11:15–11:45	Role of atovaquone-proguanil and artesunate+atovaquone-proguanil for the treatment of multidrug-resistant malaria in Cambodia **M. Wojnarski 30'**	→ For information and decision
11:45–12:30	**Discussion 45'**	
12:30–14:00	**Lunch**	
14:00–17:00	Formulation of TEG recommendations **A. Dondorp**	**Closed session**
17:00	Closing remarks **A. Dondorp/P. Alonso**	**Closed session**

Annex 3: List of questions

Session 1

Piperaquine resistance

- Is there sufficient evidence to confirm that *Pfplasmepsin 2-3* increased copy number is a marker of piperaquine resistance?
- Could other mutation(s), including in *Pfcrt*, be involved in piperaquine resistance?

Markers of reinfection and recrudescence for *P. falciparum*

- Can the WHO recommendation on distinguishing *P. falciparum* recrudescence from reinfection using *msp1*, *msp2*, and *glurp* be improved? Are any changes to the recommendations required?
- Are there other molecular markers/tools that could be used for this purpose? If so, what are the advantages and disadvantages compared to existing WHO recommendations?

***P. vivax* molecular markers**

- Are there reliable molecular markers that can be used to distinguish between reinfection, recrudescence, and relapse during *P. vivax* clinical trials?
- Are there validated molecular markers of *P. vivax* resistance to chloroquine, mefloquine, pyrimethamine, sulfadoxine, or other antimalarial medicines?

Session 2

Monitoring the efficacy of seasonal malaria chemotherapy (SMC)

- Were the recommendations of the last Technical Expert Group (TEG) used in the efficacy monitoring of SMC?
- Is there evidence showing that SMC deployment is causing resistance to emerge or increasing pre-existing resistance to one or both drugs used for SMC?
- If yes, has resistance affected the effectiveness of SMC interventions?

Session 3

Strategy for antimalarial drug resistance management

- Is there a need for a strategy on antimalarial drug resistance management to guide activities at the country level?
- Are the scope and components of the strategy, as presented to the TEG, appropriate?

Update on antimalarial drug efficacy and drug resistance

- What actions need to be taken by WHO and by the NMCPs in response to the *Pfkelch13* C580Y mutants reported outside the Greater Mekong Subregion (GMS)?

Triple therapies

- Is there sufficient evidence for the combination to be recommended/used in further trials?
- Are there additional investigations that need to be conducted on cardiotoxicity, in particular based on the latest ERG conclusions on the cardiotoxicity of antimalarial medicines?

Atovaquone-proguanil

- What could be the role of atovaquone-proguanil in the GMS?
- Are there any other combinations treatments that would be worth testing in the GMS?

Minutes of 5th TEG on Drug Efficacy and Response meeting

Geneva, 17-19 October 2017

MPAC meeting

Global **Malaria** Programme

World Health Organization

Background

- The TEG DER met 1-2 June 2017 in Geneva
- TEG members:

Arjen DONDORP (chair) **Mahidol-Oxford Research Unit, Thailand**	**Harald NOEDL** **Medical University of Vienna, Austria**	**Sarah VOLKMAN** **Harvard T.H. Chan School of Public Health, USA**
Kevin BAIRD **Eijkman Oxford Research Unit, Indonesia**	**Chris PLOWE** **University of Maryland, USA**	**INVITED SPEAKERS**
David FIDOCK ***Columbia University, USA***	**Frank SMITHUIS** **Medical Action Myanmar, Myanmar**	**Ingrid FELGER** **STPH, Switzerland**
Ian HASTINGS **Liverpool School of Tropical Medicine, UK**	**Siv SOVANNAROATH** **CNM, Cambodia**	**Paul MILLIGAN** **LSHTM, UK**
Didier MENARD **Pasteur Institute of Cambodia, Cambodia**	**Thieu Nguyen QUANG** **NIMPE, Viet Nam**	**Mariusz WOJNARSKI** **AFRIMS, Thailand**
Daouda NDIAYE ***Université Cheikh Anta Diop, Senegal***	***Neena VALECHA*** ***NIMR, India***	

- Topics covered by this meeting:
  - molecular markers
  - seasonal malaria chemoprevention
  - prevention and treatment of mdr malaria

Molecular markers

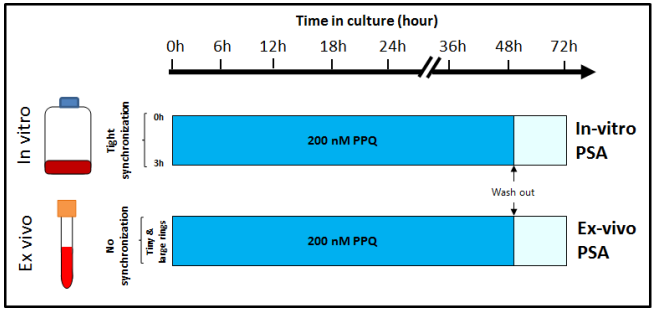
Molecular markers

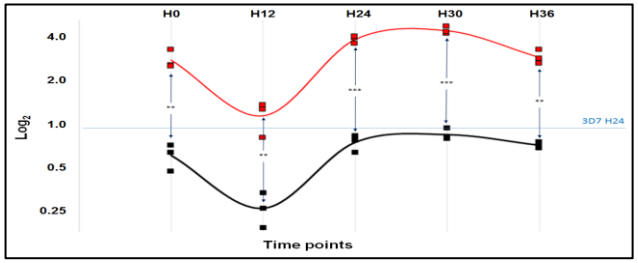
- Molecular marker(s) of piperaquine resistance;
- Molecular markers to distinguish reinfection from recrudescence in *P. falciparum* TES;
- Molecular markers of *P. vivax* – reinfection vs recrudescence and drug resistance.

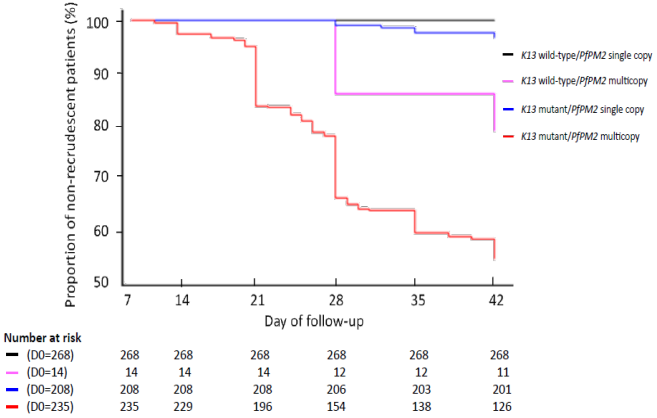
Molecular markers: piperaquine resistance

Validation of *Plasmepsin* 2-3 was done in stepwise manner:

- Validation of a new in vitro test piperaquine survival assay;
- Next-generation sequencing performed on 8 piperaquine-sensitive and 24 piperaquine-resistant parasites detecting *Pfplasmepsin 2* and *3* genes;
- Demonstration that amplification of these gene leads to overexpression of *Pfplasmepsin 2-3* mRNA;
- Correlation between increased *Pfplasmepsin 2-3* copy number and clinical failure;
- To fully validate *Pfplasmepsin 2-3* copy number as a molecular marker of piperaquine resistance, genome edited P. falciparum with single/multicopy *Pfplasmepsin 2-3* would be an invaluable tool.



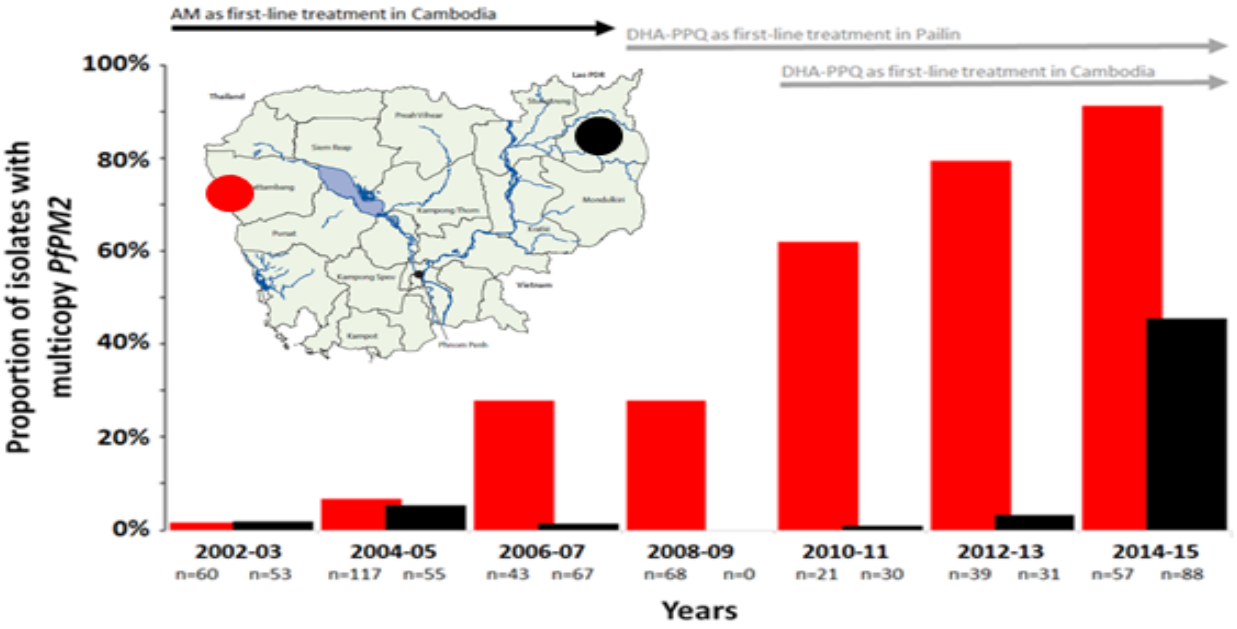




Molecular markers: piperaquine resistance

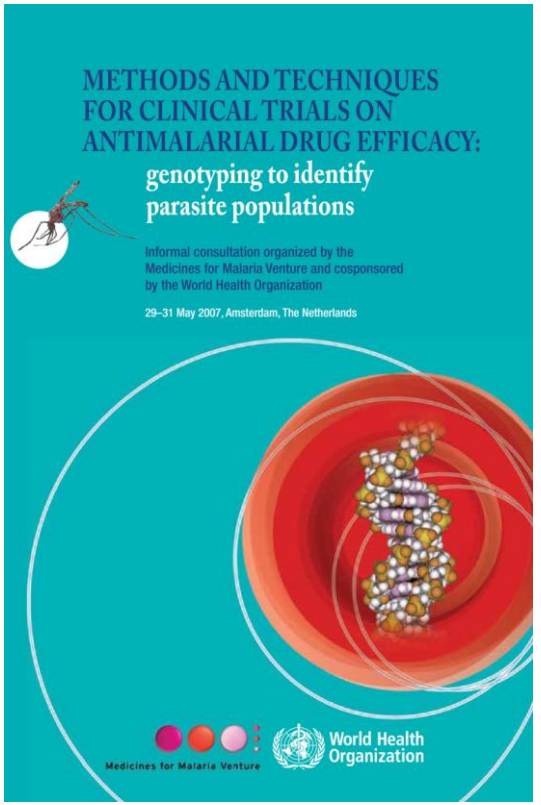
Recommendations

- There is sufficient evidence to confirm *Pfplasmepsin 2-3* increased copy number as a marker of piperaquine resistance in GMS.
- *Pfplasmepsin 2-3* increased copy number should be incorporated into surveillance and monitoring activities globally where piperaquine is being used or considered for use.
- Although other mutations may be involved in piperaquine resistance, including novel *Pfcrt* mutations (H97Y, F145I, M343L, and G353V), these require further research and validation before recommendations can be made.



Molecular markers: reinfection vs recrudescence

- In 2007, WHO published guidance on genotyping to identify parasite populations for clinical trials on antimalarial efficacy
- Recommendations were to compare *P. falciparum* parasite genotypes sequentially in pre- (day 0) and post-treatment samples (day X of treatment failure) using *msp1*, *msp2*, and *glurp* as markers of new infection vs. recrudescence.
- Issues that have been identified include:
  - poor quality of PCR execution and analysis (especially with respect to reading the agarose gels);
  - PCR bias towards short fragments;
  - template competition; and
  - limitations in the use of the sequential decision algorithm for deciding on recrudescence or reinfection, particularly in high transmission areas where multiplicity of infections is high and many coinfection clones compete with each other during PCR amplification.



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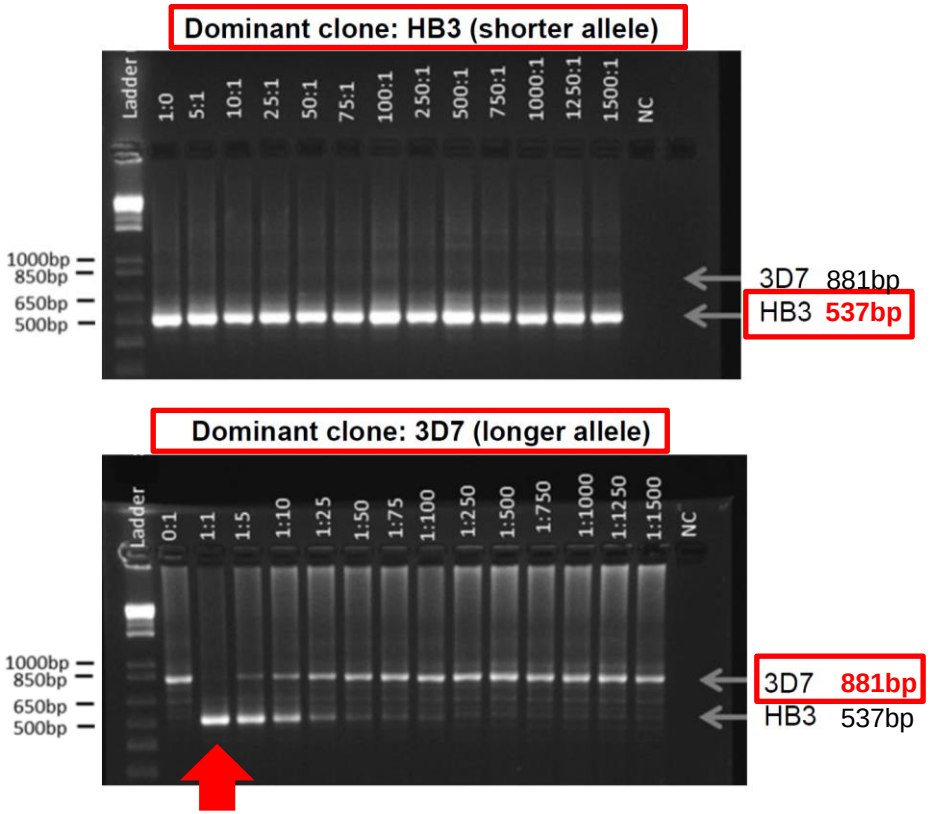
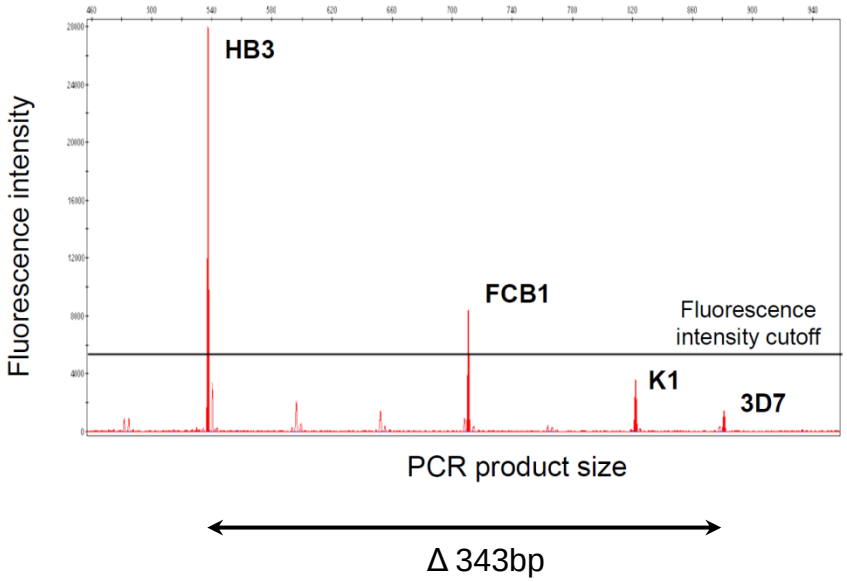
Template competition in *glurp* PCR

Swiss TPH

2-strain mixtures: different ratios



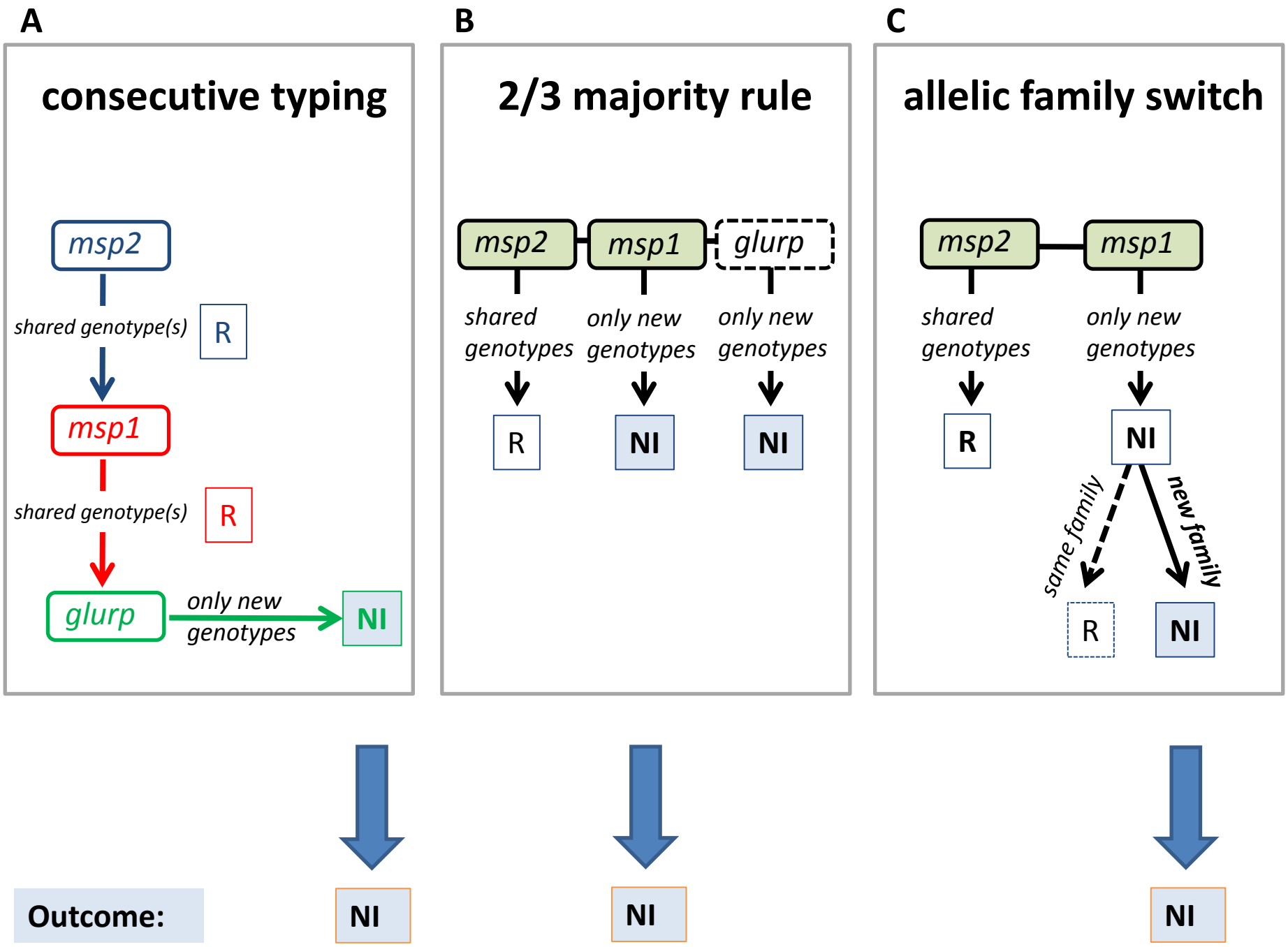
4-strain mixtures in ratio 1:1:1:1



Marker *glurp* is the least useful!

Limitations of marker *glurp*:

Longest allele sizes	→ increased competition
Only 1 allelic family	→ direct competition between all alleles
Prone to stutter peaks	→ requires increased cut-off


A
consecutive typing
msp2
shared genotype(s)
R
msp1
shared genotype(s)
R
glurp
only new genotypes
NI
B
2/3 majority rule
msp2
msp1
glurp
shared genotypes
only new genotypes
only new genotypes
R
NI
NI
C
allelic family switch
msp2
msp1
shared genotypes
only new genotypes
R
NI
same family
new family
R
NI
Outcome:
NI
NI
NI

Molecular markers: reinfection vs recrudescence

Recommendations

- The use of capillary electrophoresis for *msp1*, *msp2*, and *glurp* assessment should be promoted;
- If *msp1* and *msp2* yield congruent results, this result should be reported as the overall result of the genotyping. Where there is a discrepancy between the outcomes of markers *msp1* and *msp2*, a third marker should be genotyped (*glurp* or another validated highly diverse gene).
- In terms of assessing new techniques for distinguishing recrudescence from reinfection
  - WHO will provide samples and data from clinical studies (high and low transmission area);
  - Samples will be analyzed using results from barcoding and amplicon sequencing along with the current length polymorphism approach;
  - Results will be incorporated into the planned modelling studies and new algorithms for interpreting data will be compared for their best fit to simulated data.

Molecular markers: *P. vivax* molecular markers

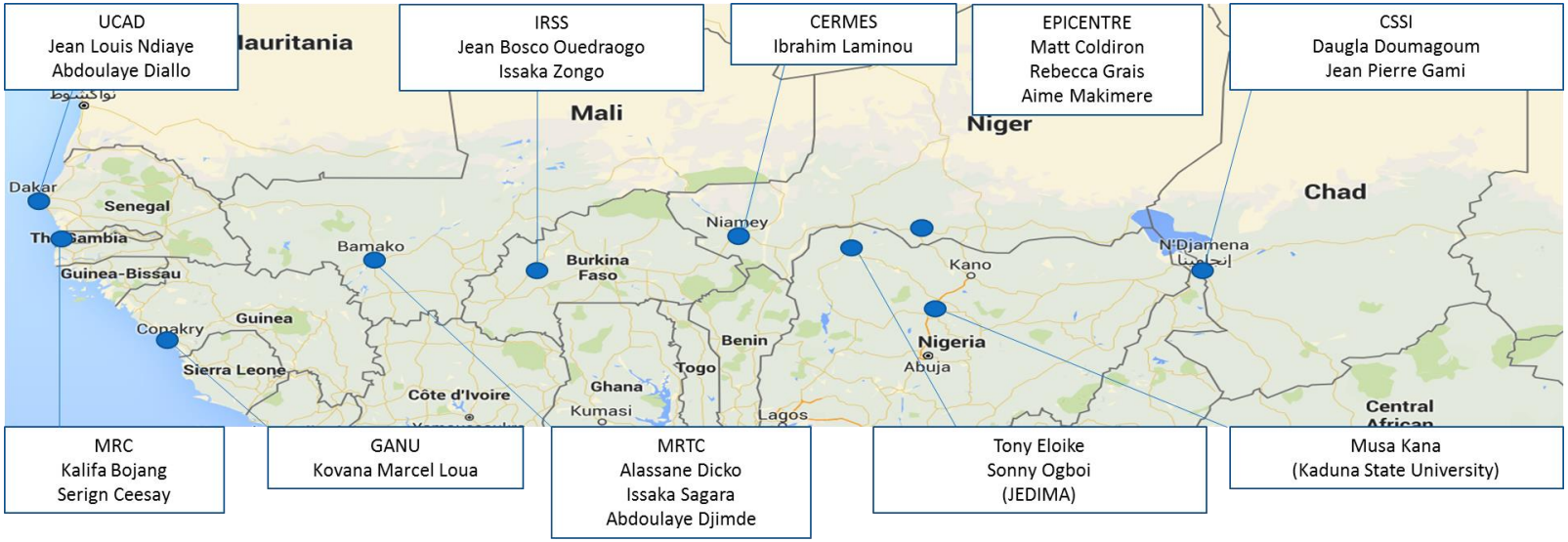
Recommendations

- There are no markers that can be used to differentiate between recrudescence, relapse, and reinfection, which makes it difficult to interpret primaquine efficacy and blood stage resistance studies;
- There are no molecular markers of *P. vivax* resistance to chloroquine, mefloquine, or primaquine. Only markers of pyrimethamine and sulfadoxine resistance have been validated, although that treatment is not recommended for acute vivax malaria under almost all circumstances.
- Clinical trials of therapies for acute vivax malaria with robust therapeutic response phenotyping protocols are needed in order to inform the search for much-needed validated molecular markers of resistance.
- Low/intermediate CYP2D6 activity has predictive value for recurrent *P. vivax* infections treated with effective blood schizontocides and primaquine. CYP2D6 genotyping should be included in primaquine clinical trials.

Seasonal malaria chemoprevention

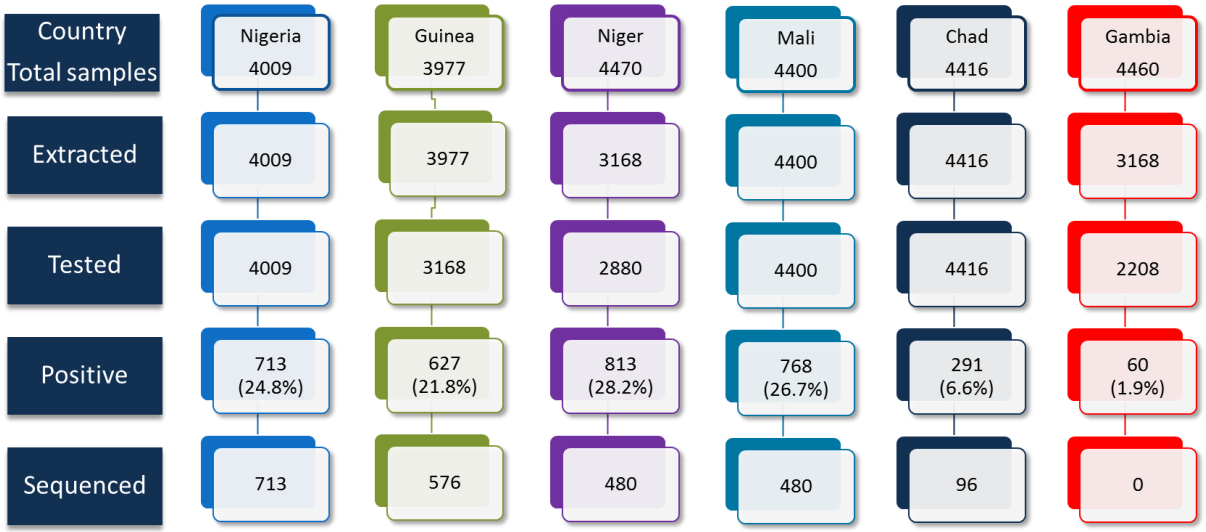
Seasonal malaria chemoprevention

- Recommended by WHO since March 2012, for children aged 3 to 59 months living in areas of highly seasonal malaria transmission in the sub-Sahel regions of Africa. Now implemented in 12 countries.
- SMC is provided for children up to 10 years of age in some areas (Senegal, parts of Mali). In parts of northern Mali, SMC is provided for all ages.
- The scale-up of SMC in 2015 and 2016 was organised largely through the ACCESS-SMC project, funded by UNITAID, in 7 countries (Burkina Faso, Chad, Gambia, Guinea, Mali, Niger, Nigeria). Similar monitoring methods in Senegal.



Seasonal malaria chemoprevention

- In 2015, baseline community surveys to monitor drug-resistance markers were conducted in areas that were yet to start SMC (with the exception of Gambia, which started SMC in 2014) in children under 5 and those aged 10–30 years.
- A total of 2000 samples were collected in each group in each area, with a total target sample size of 28 000.
- Markers were *Pfcrt* (CVMNK, CVIET, and SVMNT), *Pfmdr1* (86, 184, and 1246), *Pfdhfr* (51, 59, and 108), and *Pfdhps* (431, 436, 437, 540, 581, and 613).
- Of the 21 024 samples tested, 3448 (16.4%) were *P. falciparum* positive and 2324 have been genotyped so far.



Seasonal malaria chemoprevention

- Four samples (0.14%), all from Niger, carried pfmdr1_YY but only one had CVMNK/CVIET.
- Eight samples (0.33%), (7 from Guinea and one from Niger) carried *Pfdhfr* triple and *Pfdhps* double mutations (437+540). None of these samples carried *Pfpfmdr1* YY.
- Low frequencies of mutations associated with SP and AQ resistant genotypes.
- Prevalence of AQ markers reflects the drug combinations most used for first line malaria treatment in recent years.
- The only trends observed so far have been from Mali,
  - molecular markers of sulfadoxine-pyrimethamine resistance increased after SMC: *Pfdhfr-Pfdhps* quintuple mutant genotype increased from 1.6% to 7.1% ($p = 0.02$);
  - prevalence of *Pfmdr1* 86Y decreased from 26.7% to 15.3% ($p = 0.04$), with no change for *Pfcrt* K76T.
- Data from Mali suggest that the risk of developing drug resistance is higher with sulfadoxine-pyrimethamine than with amodiaquine, but AL may be deterring the development of amodiaquine resistance.

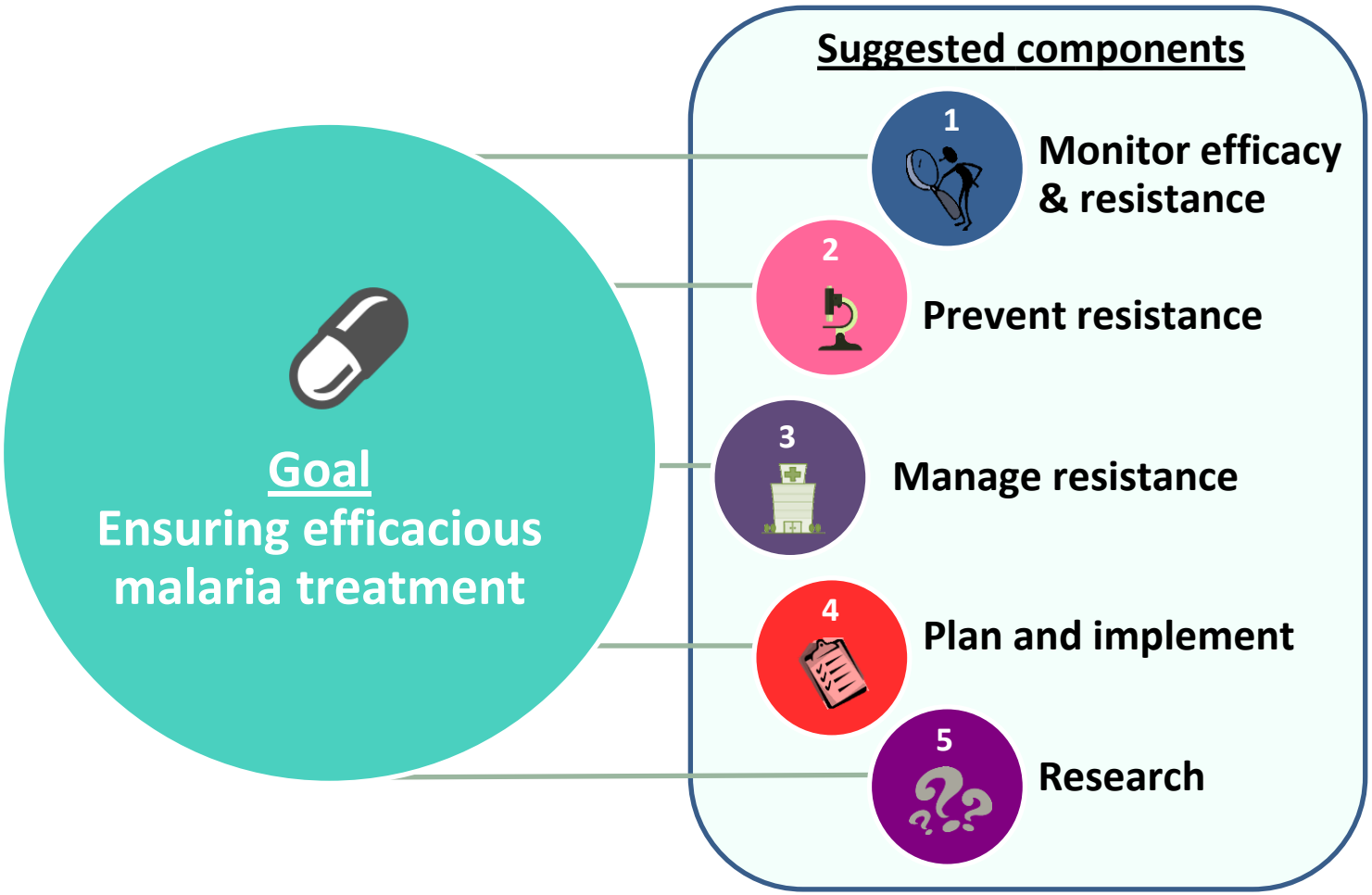
Prevention and treatment of mdr malaria

- New strategy for drug resistance management;
- Update on drug resistance;
- Triple therapies in the GMS;
- Atovaquone-proguanil in GMS;
- Update on spreading lineage in GMS.

Rationale for the development of a new strategy for drug resistance management

- Recent WHO guidance has focused on artemisinin resistance:
  - Global plan for artemisinin resistance containment (GPARC) released in 2011.
  - Emergency response to artemisinin resistance in the Greater Mekong subregion, Regional framework for action 2013-2015 (ERAR) released in 2013.
- Since GPARC, understanding of artemisinin resistance and resistance to ACT partner drugs has improved.
- Drug resistance is a challenge not only for the artemisinin-based treatments.
- Countries have been requesting concrete guidance for drug resistance management.

Suggested components of the strategy

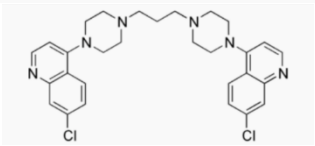


Recommendations

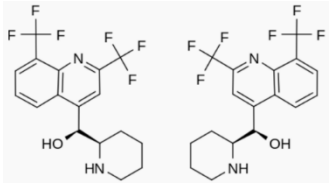
- TEG agreed that it would be valuable to have a new strategy for antimalarial drug resistance management;
- The scope and components of the strategy presented were considered appropriate and should include the following:
  - Scenario-planning, for instance in case of outbreaks of falciparum malaria in areas with multidrug resistance;
  - Guidance on *P. vivax* resistance;
  - New information and approaches since the GPARC;
  - Distinct scenario-planning for different resistance situations;
  - Consideration of all interventions using antimalarial drugs, their potential impact on resistance development, and actions that might mitigate this risk;
  - Measures for containment across borders;
  - Guidance on the management of suspected and confirmed treatment failures, including diagnostics and alternative treatments that can be used in remote or resource-poor areas.
- An ideal format would include a generic section building on what is in the GPARC and what has been learned more recently, plus scenarios that can change over time as new evidence and tools become available.

TACT: DHA-piperaquine + mefloquine

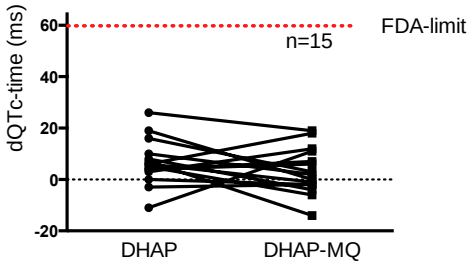
Piperaquine



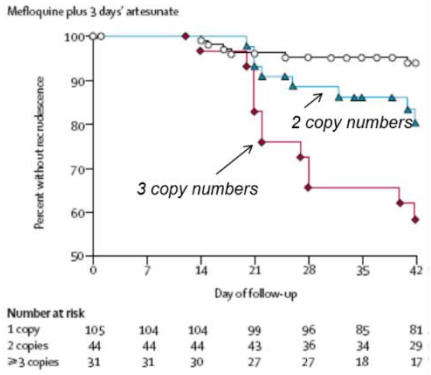
Mefloquine



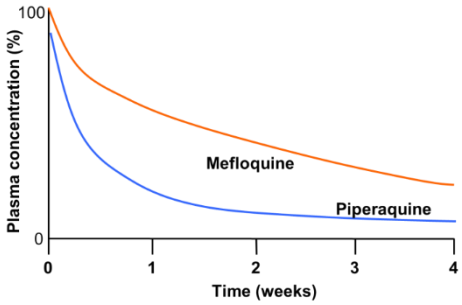
No interaction re QTc time



Possible counter-acting resistance mechanisms



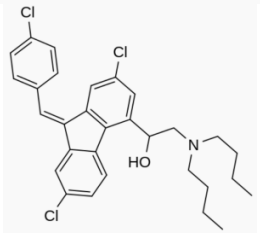
Reasonably matching PK-profiles



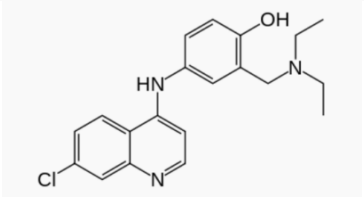
Price et al. Lancet 2004

TACT: Artemether-lumefantrine + amodiaquine

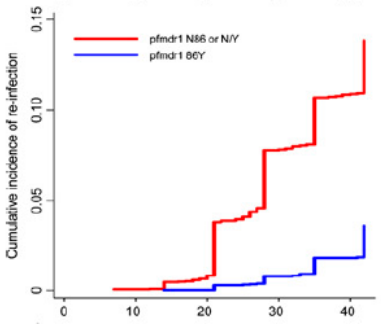
Lumefantrine



Amodiaquine



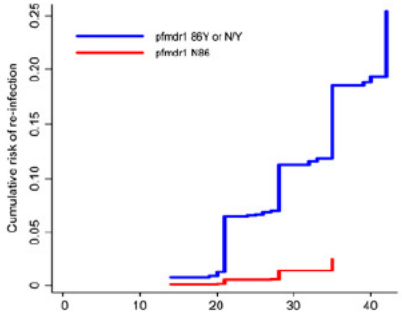
Selection after Artemether-Lumefantrine



Treatment failure

PfMDR1
N86Y

Selection after Artesunate-amodiaquine



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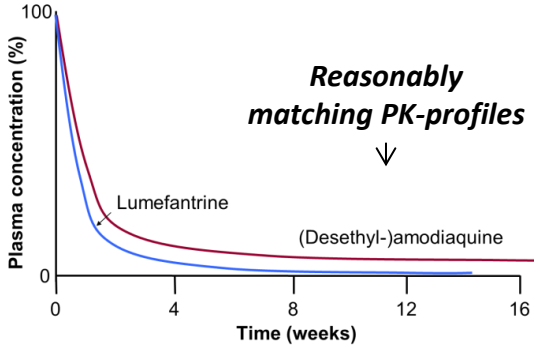
Counter-acting resistance mechanisms

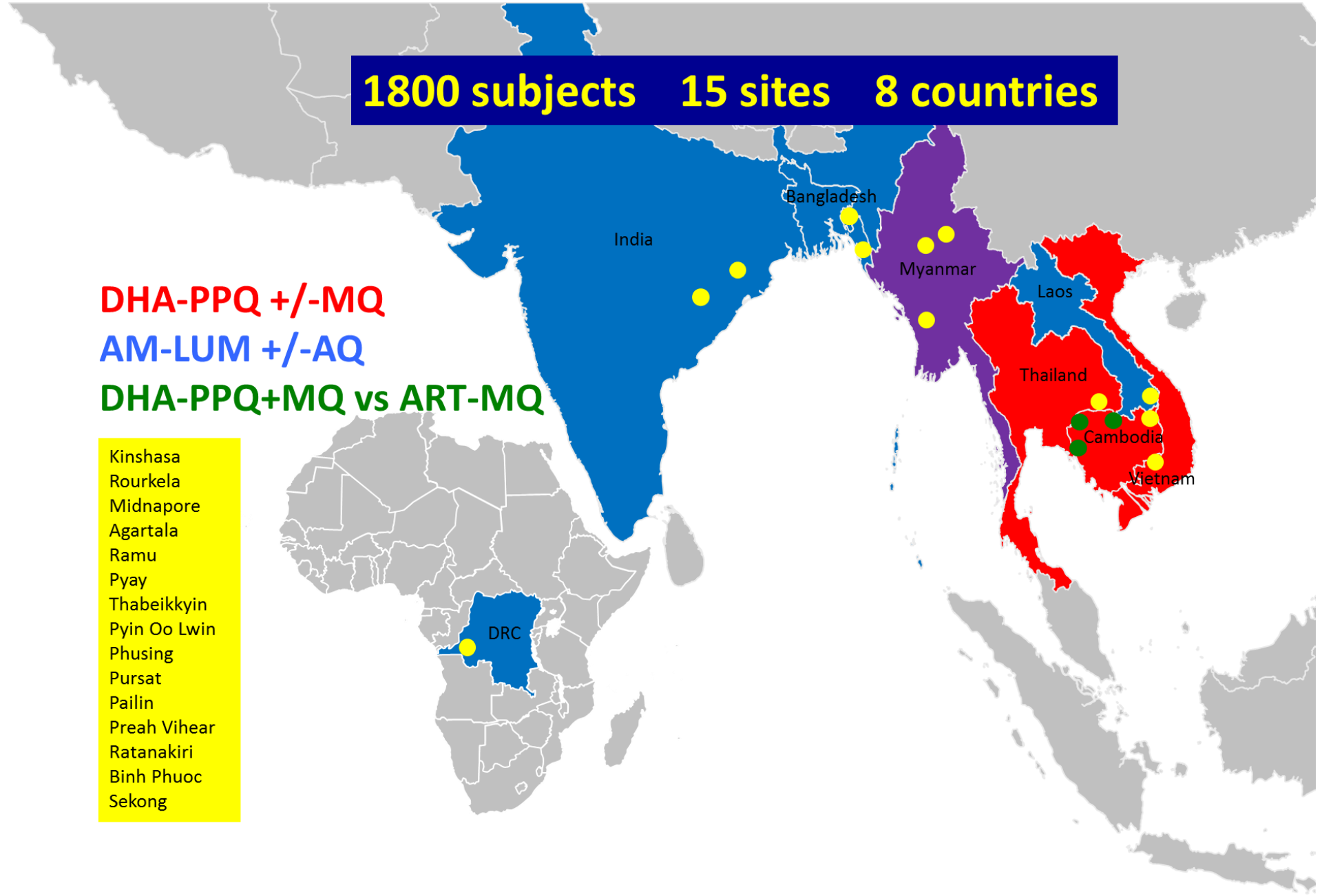


Reasonably matching PK-profiles

↓

Venkatesan et al.
Am J Trop Med Hyg 2014

wellcome trust MORU Mahidol Oxford
WELLCOME TRUST - MAHIDOL UNIVERSITY - OXFORD
TROPICAL MEDICINE RESEARCH PROGRAMME


1800 subjects 15 sites 8 countries
India
Bangladesh
Myanmar
Laos
Thailand
Cambodia
Vietnam
DRC
DHA-PPQ +/-MQ
AM-LUM +/-AQ
DHA-PPQ+MQ vs ART-MQ
Kinshasa
Rourkela
Midnapore
Agartala
Ramu
Pyay
Thabeikkyin
Pyin Oo Lwin
Phusing
Pursat
Pailin
Preah Vihear
Ratanakiri
Binh Phuoc
Sekong

42-day efficacy

Country	Site name	DHA-PPQ	ART-MQ	DHA-PQ+MQ	p value
		Recurrent infections	Recurrent infections	Recurrent infections	DP vs DP+MQ
Cambodia	Pailin	5/9 (55.6%)	0/2 (0%)	0/14 (0%)	0.004
Cambodia	Pursat	6/8 (75.0%)	0/17 (0%)	0/25 (0%)	<0.001
Cambodia	Preah Vihear	NA	0/2 (0%)	0/3 (0%)	NA
Cambodia	Ratanakiri	2/7 (28.6%)	NA	0/6 (0%)	0.46
Vietnam	Binh Phuoc	8/20 (40.0%)	NA	0/22 (0%)	0.001
Thailand	Phusing	8/9 (88.9%)	NA	0/11 (0%)	<0.001
Thailand	Tha Song Yahn	NA	NA	0/1 (0%)	NA
Myanmar	Thabeikkyin	0/13 (0%)	NA	0/13 (0%)	*
Myanmar	Pyay	0/12 (0%)	NA	0/11 (0%)	*

PCR uncorrected

- *No recurrent infections in both arms*

42-day efficacy

Country	*Site name*	*AL*	*AL+AQ*	*p value*
		Recurrent infections	*Recurrent infections*	*AL vs AL+AQ*
Myanmar	Pyin Oo Lwin	0/6	0/10	*
Laos	Sekong	1/6	0/5	1.00
Bangladesh	Ramu	2/44	0/45	0.24
India	Agartala	0/9	0/10	*
India	Midnapore	0/5	0/5	*
India	Rourkela	3/15	0/14	0.22
DRC	Kinshasa	15/60	13/60	0.83

Likely re-infections

PCR uncorrected

Mahidol Oxford

WELLCOME TRUST - MAHIDOL UNIVERSITY-OXFORD
TROPICAL MEDICINE RESEARCH PROGRAMME

- *No recurrent infections in both arms*

SAEs

DHA-PPQ

- QTc-interval prolongation >60 ms (X4)
- Pneumonia

AS-MQ

- Grade 4 increase AST/ALT

AL

- QTc-interval prolongation >60 ms (X1)
- Febrile convulsion (D0) (AL)
- Suspicion hemolytic anemia/dilution

DHA-PPQ+MQ

- QTc-interval prolongation >60 ms (X2)
- Post Malarial Neurological Syndrome (convulsion 23 y/o male after MQ)
- Hyponatremia/withdrawal symptoms
- Severe malaria (at baseline) (X2)
- Cellulitis leg (after scratching)

AL+AQ

- QTc-interval prolongation >60 ms (X1)
- Bradycardia (AL+AQ, hypokalemia)
- Grade 4 creatinine increase D28

Recommendations

- Although TRAC 2 data are preliminary, the data support the testing of triple therapies as a potential strategy against multidrug-resistant *P. falciparum*.
- Nevertheless the following concerns were raised: use of mefloquine as a monotherapy, the prevalence of double-mutant (mefloquine and piperaquine) in Cambodia, the additional pressure on the mdr lineage circulating in the GMS by continuous use of DHA-piperaquine and the potential cardiotoxicity.
- Artemether-lumefantrine+ amodiaquine was considered as more appropriate and was recommended for testing in Cambodia and Viet Nam.
- Given the concern over QTc interval prolongation interval and the issues regarding the measurement of changes in QTc as malaria symptoms resolve, further analysis of QTc using alternative methods was requested.
- An alternative treatment option for multidrug-resistant *P. falciparum* is to use two sequential artemisinin-based combination therapies (ACTs).

Efficacy by Treatment Arm

Regimen		PCR Correction	Efficacy Rate (%) Day 28 (95% CI)	Efficacy Rate (%) Day 42 (95% CI)
mITT	Atovaquone-Proguanil	Yes	98.9 (92.5-99.8)	**90.4** (81.6-95.1)
		No	98.9 (92.5-99.8)	89.1 (80.0-94.2)
	Artesunate-Atovaquone-Proguanil	Yes	100.0 (-)	**92.0** (82.9-96.3)
		No	100.0 (-)	89.4 (80.0-94.6)
ACPR (PP)	Atovaquone-Proguanil	Yes	98.7 (91.0-99.8)	**89.5** (80.1-94.6)
		No	98.7 (91.1-99.8)	88.3 (78.7-93.7)
	Artesunate-Atovaquone-Proguanil	Yes	100.0 (-)	**91.7** (82.4-96.2)
		No	100.0 (-)	89.2 (79.5-94.4)

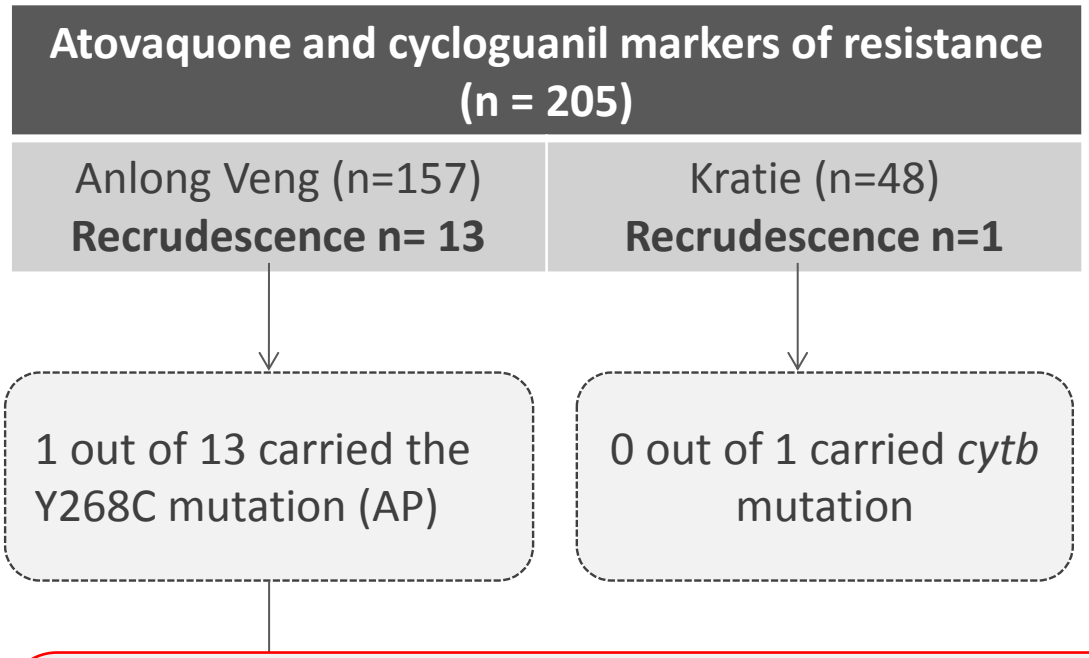
Efficacy by Location in Cambodia

Anlong Veng	Regimen	PCR Correction	Efficacy Rate (%) Day 28 (95% CI)	Efficacy Rate (%) Day 42 (95% CI)
mITT	AP	Yes	98.6 (90.3-99.8)	**87.8** (77.1-93.7)
		No	98.6 (90.3-99.8)	86.2 (75.1-92.6)
	ASAP	Yes	100.0 (-)	**91.4** (80.5-96.3)
		No	100.0 (-)	88.1 (76.6-94.1)

Kratie	Regimen	PCR Correction	Efficacy Rate (%) Day 28 (95% CI)	Efficacy Rate (%) Day 42 (95% CI)
mITT	AP	Yes	100 (-)	**100** (-)
		No	100 (-)	100 (-)
	ASAP	Yes	100.0 (-)	**93.8** (63.2-99.1)
		No	100.0 (-)	93.8 (63.2-99.1)

Cause of treatment failure (n=14)

- Only **1 subject** identified with *Pfcytb* mutation on DR



Pf cytochrome b (*cytb*) Y268 locus by **Sanger sequencing**

Suspected ATQ resistance through treatment

Amplicon deep sequencing targeting cytb confirmed the presence of the Y268C mutation in 99.6% of the sequence reads at recrudescence, but did not detect the mutation pre-treatment or 24 hours into treatment, even at a minor allele frequency down to 0.25% (33,267 and 6,047 reads at D0 and D1)

Recommendations

- In the GMS, there may be a role for atovaquone-proguanil in combination with an ACT;
- AS-MQ+AP and AS-PY+AP are two options for testing;
- Further studies are required to validate mutations as a clinically relevant molecular marker of atovaquone resistance. There may be other mutations contributing to resistance besides the Pfcytb mutation at position 268;
- Until there is stronger evidence that a P. falciparum Pfcytb Y268C/N/S mutant is not transmissible, it cannot be concluded that atovaquone resistance is not transmissible.

Prevalence of *Pfplasmepsin 2-3* increased copy number

Year	Countries	Prevalence	Study
2013	Comoros	4/46 (8.7%)	TES
2015	Mozambique	0/87 (0%)	TES
2015	Mozambique	1/88 (1.1%)	TES
2015	Mozambique	1/89 (1.1%)	TES
2015	Mozambique	2/87 (2.3%)	TES
2015	Mozambique	3/61 (4.9%)	Pre-MDA
2016	Mozambique	1/19 (5.3%)	Post-MDA

Recommendations

- presence of multicopy *Pfplasmepsin 2-3* in Africa is a potential concern in terms of the use of DHA-PIP;
- additional information is required regarding the in vivo and ex vivo piperaquine-resistant phenotype in African parasites;
- additional African data are needed to assess the relationship between DHA-PIP treatment failures and molecular markers (*Pfkelch13*, *Pfplasmepsin 2-3*, and *Pfcrt*).

Update on antimalarial drug efficacy and drug resistance

Article

- Lu F et al. Emergence of Indigenous Artemisinin-Resistant Plasmodium falciparum in Africa. N Engl J Med. 2017 Mar 9;376(10):991-993.

Summary

- Chinese worker returning from Equatorial Guinea and developing a malaria attack in China treated successfully with DHA-pipearquine. Day 3 parasitaemia: 40/ml (1/200 WBC); RSA0-3h survival rate ≈ 2%, PfKelch13: M579I.

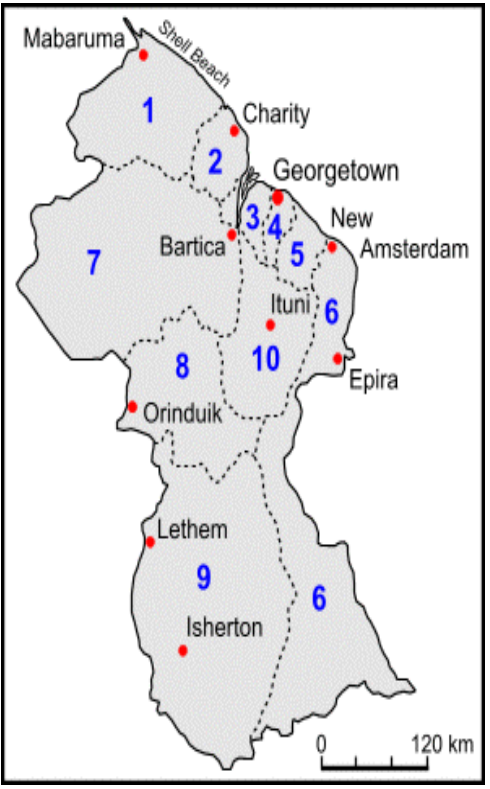
Response to the editor

- The WHO criteria for calling a PfKelch13 mutation confirmed include: a significant association between the mutation and delayed clearance in at least 20 clinical cases, and RSA0-3h survival rate >1% in at least 5 individual isolates or culture-adapted recombinant isogenic parasite lines, produced using transfection and gene editing techniques.

Recommendation

- The TEG recommends that all putative Pfkelch13 mutants conferring artemisinin resistance be independently verified as being associated with resistance both in genetic studies and in the RSA, ideally before publication claiming such association.

Update on antimalarial drug efficacy and drug resistance

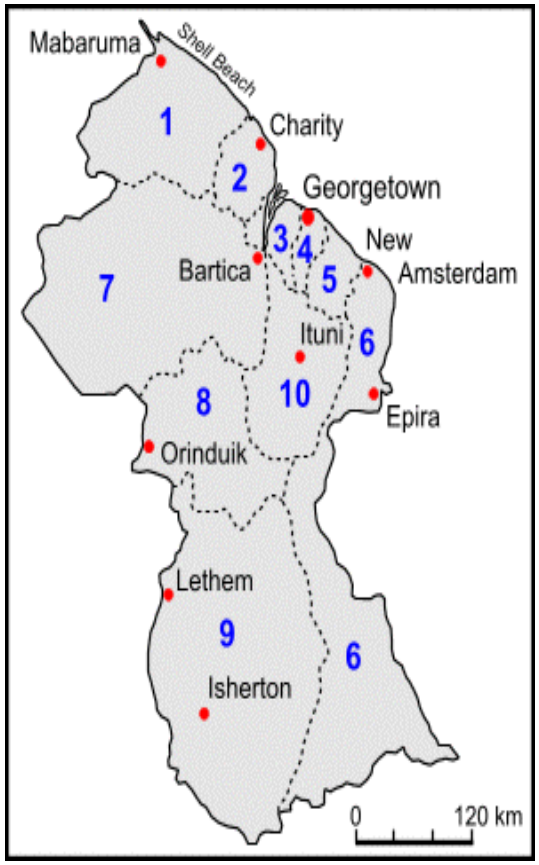


- Collaboration with Institut Pasteur Cayenne
- Sample collected in 2010 for HRP2 survey; 5 samples carried the mutant C580Y (**4/5 from zone 7** and **1/5 zone 1**);
- All five samples had similar K13 flanking microsatellite profiles and were different to the ones observed in Southeast Asia;
- June-Nov 2014: 7-day artesunate trial (4 mg/kg/day) + primaquine single dose; 2% day-3 positivity rate; 100% efficacy and 100% of K13 wild type; N = 50 (26% from zone 1; 54% zone 7; 16% zone 8)
- Survey conducted in 2016 (n = 691) confirmed presence of C580Y mainly in zone 1; QA/QC and flanking microsatellite confirmed South American origin.

Recommendations

- planned activities (TES and survey) to investigate Pfkelch13 C580Y in South America are sufficient.
- whole genome sequencing may be useful to examine backbone mutations and resistant parasites should be collected for culture adaption.

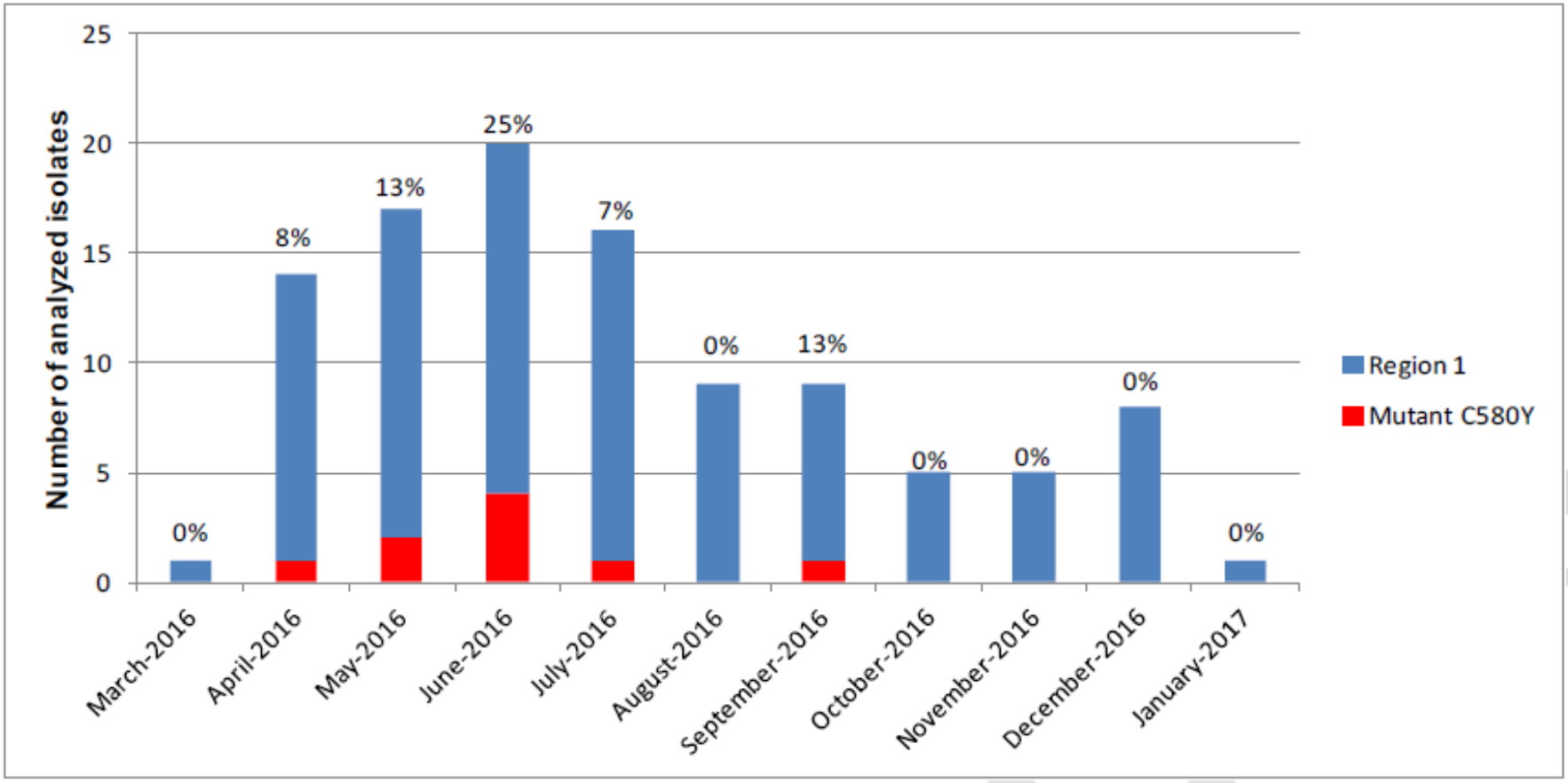
Update on antimalarial drug efficacy and drug resistance



	C580	580Y	Total	%
Region 1	87	9	96	**9.4**
Region 2	2	0	2	0
Region 3	8	0	8	0
Region 7	474	3	477	**0.6**
Region 8	94	1	95	1
Region 9	2	0	2	0
Venezuela	11	0	11	0
Total	**678**	**13**	**691**	**1.9**

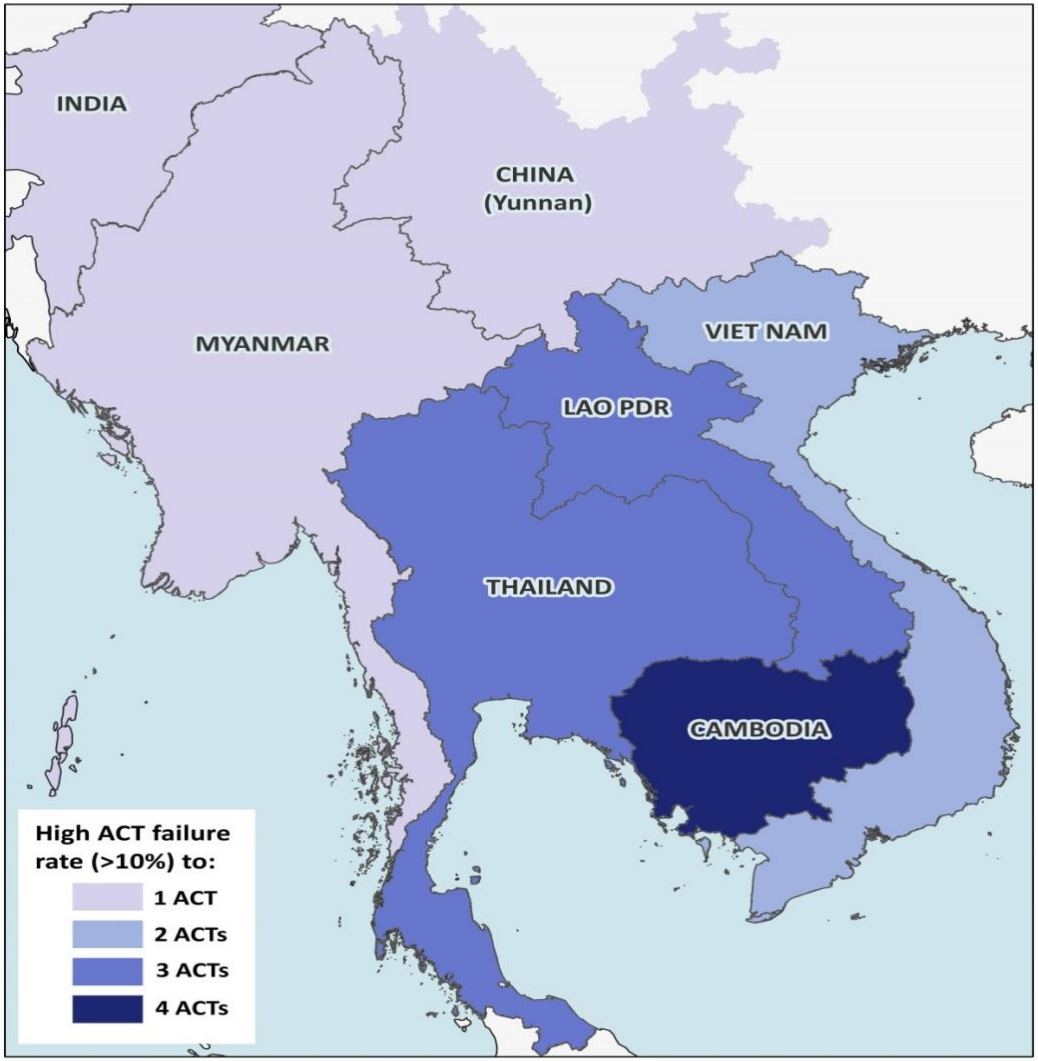
Prevalence of *Pfkelch13* C580Y in Guyana

Update on antimalarial drug efficacy and drug resistance



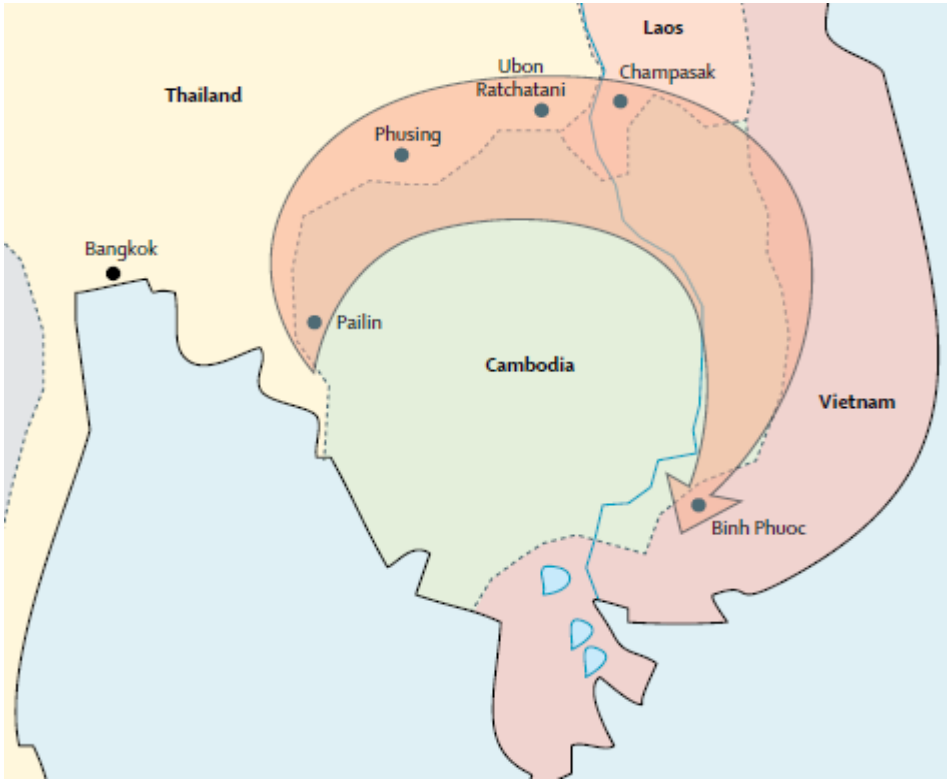
Prevalence of *PfK13* C580Y in Guyana over time

Update on antimalarial drug efficacy and drug resistance



Update on antimalarial drug efficacy and drug resistance

Spread of s single multidrug resistant malaria parasite lineage (*Pf Pailin*) to Viet Nam



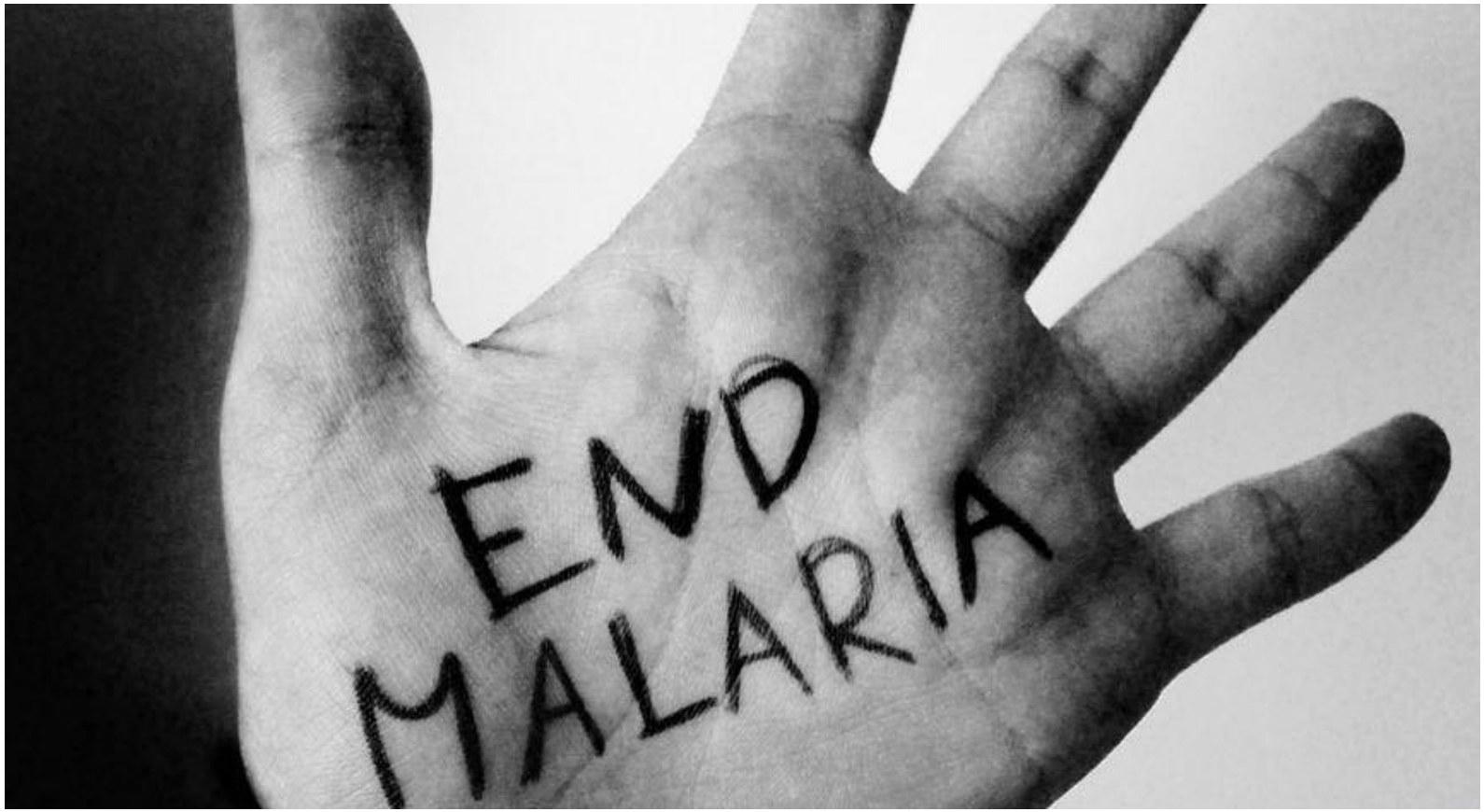
Imwong et al. 2017 Lancet Inf Dis.

- The recent Lancet article created a significant attention of the press on “resistant malaria”
- The spread of resistant parasites across the region underscores the importance of cross-border collaboration

Update on antimalarial drug efficacy and drug resistance

- "Super malaria" has not been adequately defined; the scientific and public health community does not recognize this term
- The problem of multidrug resistance in the Greater Mekong Subregion (GMS), including in Viet Nam, has been well known to WHO for a number of years. In 2014 and 2015, 4 studies conducted by Viet Nam's NMCP, in collaboration with WHO, already demonstrated high treatment failure rates with dihydroartemisinin-piperaquine ranging from about 26% to 46%.
- In September 2016, Viet Nam's NMCP changed its policy for first-line treatment of malaria, replacing DP (in provinces where DP is failing) with artesunate-mefloquine.
- The NMCP, under the auspices of WHO, has been testing a new artemisinin-based combination therapy (ACT): artesunate-pyronaridine;
- In December 2016, at the request of the Malaria Policy Advisory Committee (MPAC), an Evidence Review Group (ERG) analysed the body of evidence on the emergence and spread of multidrug-resistant *P. falciparum* in the GMS over the last decade.
- A public health emergency of international concern (PHEIC), as defined in the International Health Regulations (IHR), is an "extraordinary event." These regulations were designed to address acute (as opposed to chronic) public health conditions.

Thank you for your attention



Global response plan for *pfhrp2/3* deletions

Jane Cunningham

MPAC 17-19 October, 2017

Global **Malaria** Programme

World Health Organization

The global response plan

for *pfhrp2/3* mutations that limit the effectiveness of HRP2-based RDTs comprises a global framework to support national malaria control programmes and their implementing partners to address this problem pragmatically.

Objectives

- define the frequency and distribution of diagnostically relevant *pf-hrp2/3* mutations in circulating *P. falciparum* strains;
- provide concrete guidance to countries on malaria diagnosis and treatment in settings where such mutations are found to be frequent;
- identify gaps in knowledge about the genesis and spread of strains with *pfhrp2* and/or *pfhrp3* deletions and the actions required to develop new, accurate tests for malaria based on alternative target antigens; and
- coordinate advocacy and communication with donors, policy-makers, test developers, research agencies, technical partners and disease control programmes to assist in planning.

Background

- Overview of RDTs – how they work, targets, quality assurance, utilization/market trends
- Evolution of *pfhrp2/3* deletion mutants & key conclusions:
  - There are clear local “hot spots” - Amazonian regions of Colombia and Peru and in Eritrea.
  - The prevalence of *P. falciparum* that do not express HRP2 varies by province in any given country.
  - *pfhrp2/3* deletion mutants can cause outbreaks which may be missed by HRP2-based RDTs ie. in Tumbes, Peru.
  - In many studies, the methods by which patients were selected resulted in overestimates of the true prevalence of *pfhrp2/3* deletion mutants eg. reporting prevalence of *pfhrp2* deletions based on total discordant samples instead of all microscopy + Pf cases.

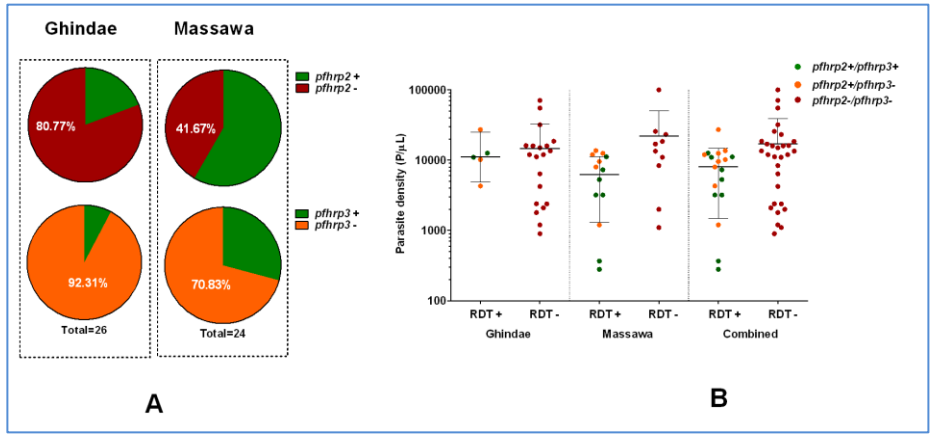
Background

- Depending on survey design, it is also possible to underestimate the prevalence of *pfhrp*2-deleted strains due to:
  - cross-reactivity of HRP2-based RDTs with HRP3
  - the circulation of strains with *pfhrp2* deletions which may be masked by co-infection with *P. falciparum* strains without such deletions (infection with more than one strain type).
- The absence of PCR amplification of *pfhrp2/3* may be due to an inadequate quantity of parasite DNA.
  - In many studies, the methods used to obtain DNA did not provide enough material to detect single-copy genes like *pfhrp2/3*.

Scenarios

NMCPs and their implementing partners:

Scenario 1: 5% local prevalence of false-negative HRP2 RDTs due to gene deletions warrants a change in testing strategy nationwide

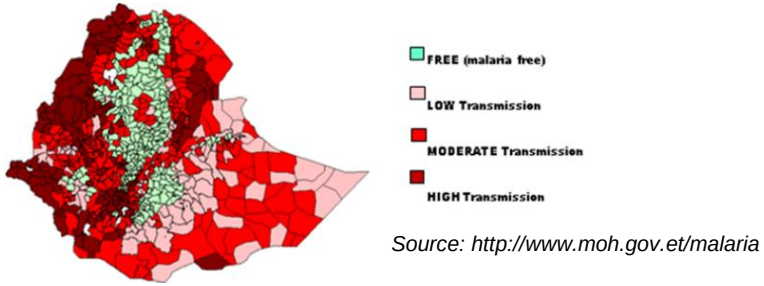


Source: Berhane et al submitted for publication

Scenario 2: suspected false negative RDTs – collect initial evidence (HRP2 RDT neg; Pf microscopy + or pf-pLDH +) and if molecular analysis confirms pfhrp2 deletion - implement survey

Case Report: A Case of *Plasmodium falciparum hrp2* and *hrp3* Gene Mutation in Bangladesh — Am J Trop Med Hyg. 2017 Aug 7

Scenario 3: national assessment of prevalence of *pfhrp2/3* deletion mutants – highest priority for countries bordering those with confirmed reports



Source: http://www.moh.gov.et/malaria

Ethiopia

Survey Protocol

Box 2. Standardized protocol for assessing national prevalence of *pfhrp2/3* deletion mutants among patients with falciparum malaria

Subjects: Symptomatic patients with suspected falciparum malaria among those seen at 10 health facilities in selected provinces

Screening method: A high-quality RDT for detecting HRP2 and either microscopy or a second RDT for detecting PfpLDH

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Sample size: 370 falciparum cases per province or region will be screened with dual RDTs. Molecular analysis will then be undertaken on the samples suspected to have *pfhrp2*/3 deletions and a statistical analysis of the prevalence with 95% CI will be computed. The analysis will result in one of three outcomes per province:

Outcome 1: That the upper limit of the 95% CI does not overlap with 5%. In this case the is a high statistical confidence that *pfhrp2/3* deletion causing false negative RDT results is below 5%

Outcome 2: The lower limit of the 95% CI is above 5%. This result means that there is a high statistical confidence that *pfhrp2/3* deletion causing false negative RDT results is greater than 5%

Outcome 3: The statistical analysis shows that it is inconclusive (5% contained within the 95% CI) as to whether or not the prevalence of *pfhrp2/3* deletion causing false negative RDT results is greater than or less than 5%.

Testing location: RDT and/or microscopy testing will be performed at local health facilities, with appropriate quality control. Molecular analysis may be performed at regional or international reference laboratories.

Discussion point #1

Actions based on outcomes:

→ Repeat survey in two years

→ Change RDT nationwide and prioritize based on prevalence in other provinces

→ Depending on resources:
- Continue enrolling patients
- Repeat survey in 1 year
- Repeat survey in 2 years

World Health Organization

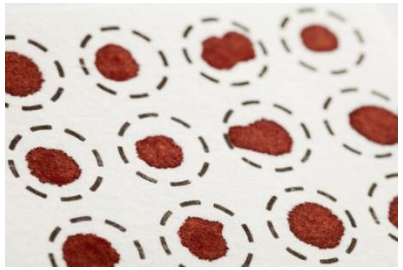
Alternative RDT options ?

- Currently very limited and further challenged by new requirements of WHO PQ in 2018
- 2 pan-only and 1 pf-pLDH + pan-pLDH combo test that meet performance criteria; only 1 of the pan-pLDH tests is prequalified
- Some tests must be assessed against *pfhrp2/3* deleted strains to determine performance ie. HRP2 and pf-pLDH on the same test line
- Performance criteria “loosened” for selection of pf-pLDH RDTs for surveys

Laboratory strengthening

- Meeting the requirement for capacity to assess suspected false-negative HRP2-based RDTs and for surveys will require quality-assured microscopy and/or staff trained and ready to use correctly non-HRP2-only RDTs that are not in routine use in the NMCP.
- Confirming the presence of genetic deletions will require sampling, labelling and preparation of dried blood spots for shipping and multiple PCR analyses in regional or international laboratories.

Estimating volume of PCR testing required



Countries with *pfhrp2* deletions reported	Neighbouring countries	Number of administrative divisions	Minimum number of falciparum cases to identify	Number of molecular assessments at 2% HRP RDT discordance	Number of molecular assessments at 5% HRP RDT discordance	Number of molecular assessments at 10% HRP RDT discordance
Mali		9	3330	67	167	666
	Mauritania	15	5550	111	278	1110
	Algeria	48	17760	355	888	3552
	Niger	16	5920	118	296	1184
	Burkina Faso	13	4810	96	241	962
	Côte d'Ivoire	14	5180	104	259	1036
	Guinea	7	2590	52	130	518
	Senegal	14	5180	104	259	1036
Total:		**136**	**50,320**	**1,007**	**2,518**	**5,032**

- A number of international reference laboratories with experience in *pfhrp2/3* molecular analysis are already collaborating with WHO
- National programmes themselves may have an interest in using or strengthening local capacity for PCR; however, the lack of PCR standardization and of a malaria molecular assay approved by a stringent regulatory authority will make comparison of results between studies and between laboratories problematic
- countries embarking on national HRP2 deletion surveys should have a molecular assessment plan that includes the capacity to ship samples internationally to collaborating laboratories with the necessary capacity and quality control.

Research

- understand the factors that drive the evolution and spread of *pfhrp*2/3 deletion mutants;
- operational and technical research to simplify the process of identifying and tracking the distribution of these strains

Research & Development

- new protein targets;
- optimization of monoclonal antibodies or other ligands, to increase their robustness, thermostability and affinity.

Coordination of the response

- many different interests involved in the discovery, development, quality control, selection, procurement, distribution, storage and use of RDTs;
- coordinating mechanism/consortium needed to provide structure for harmonized action

Discussion point #2

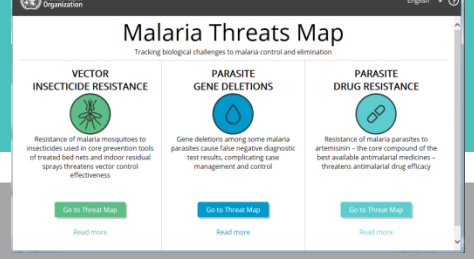
Hosted by WHO ?

Priority areas of work

Discussion point #3

- mapping the distribution and frequency of *pfhrp2/3* deletion mutants with harmonized protocols and creation of a registry of *pfhrp2/3* prevalence surveys;

Malaria Threat Maps: Data



GMP Databases

Vector insecticide resistance
1949 - 2017
bioassays, mechanisms
n = 29,137 tests

***hrp2/3* gene deletions**
1996 - 2017
Suspected and confirmed
n = 131 survey areas; 26 countries

Drug efficacy & drug resistance 2004 - 2017
TES and molecular markers
n = 2133 studies

Malaria Threats Map

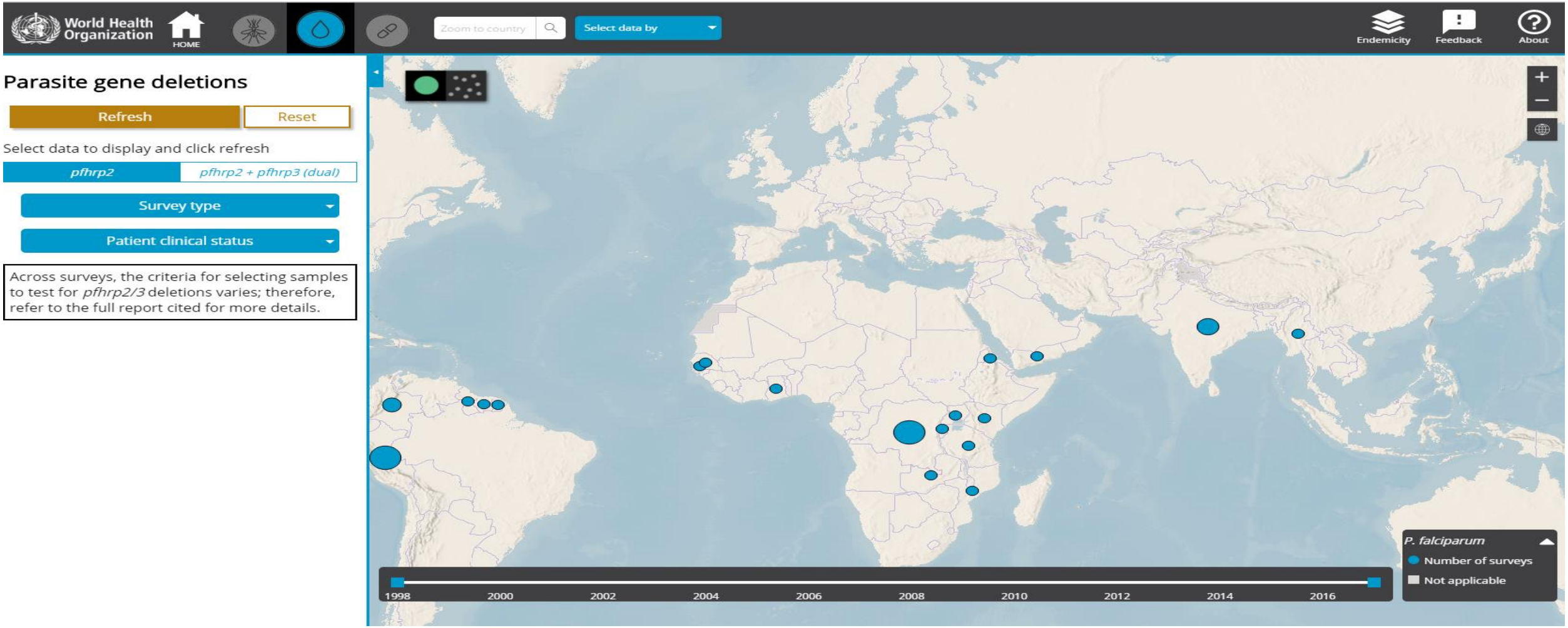
Vector insecticide resistance
2010 - 2017
bioassays, mechanisms
n = 18,712 tests

***hrp2/3* gene deletions**
1998 – 2017
Confirmed deletions, only
n= 125 survey areas; 24 countries

Drug efficacy & drug resistance 2010 - 2017
TES and molecular markers
n = 1006 studies

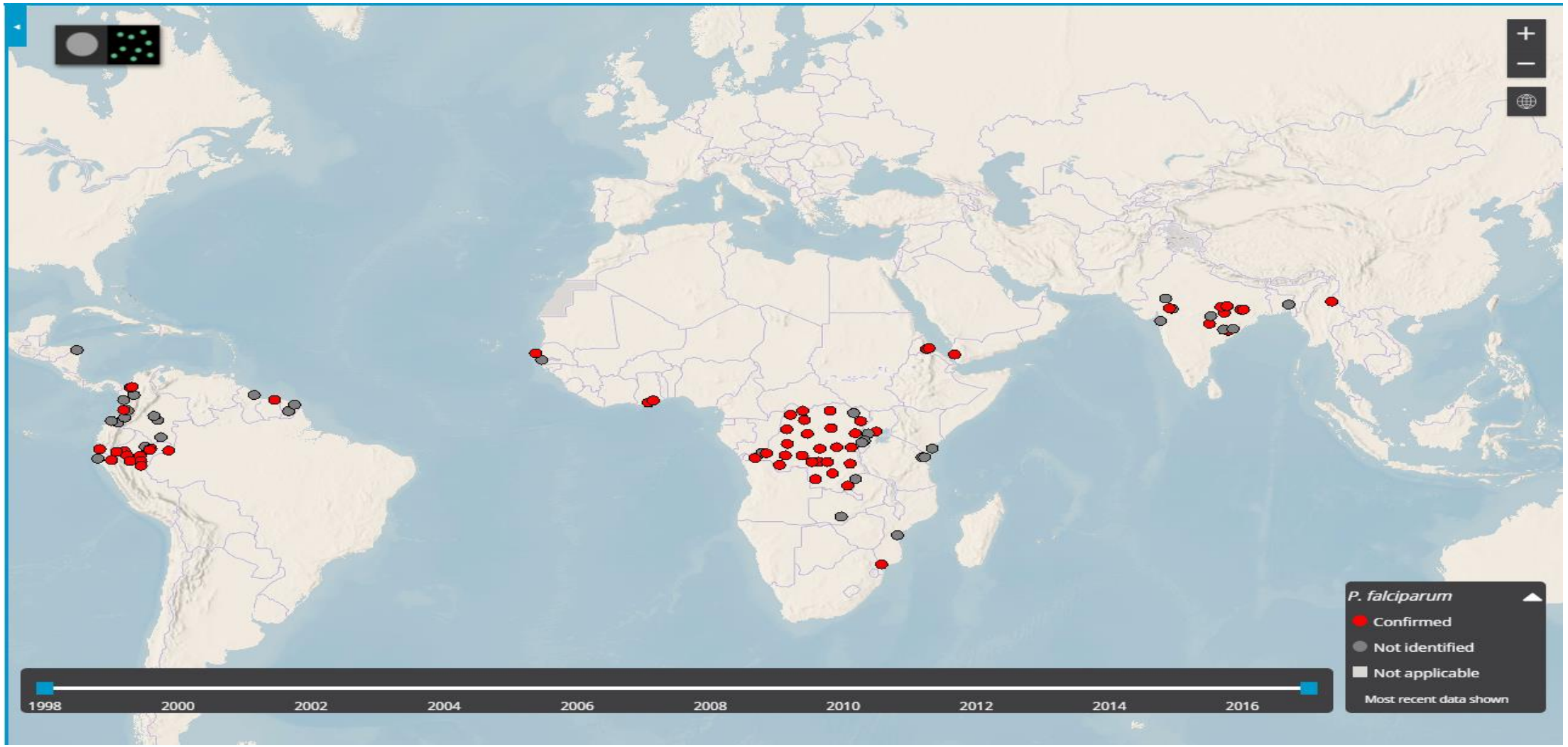
- Type of deletion
  - *pfhrp2*
  - *pfhrp2 and 3*
- Clinical status
  - symptomatic
  - asymptomatic
  - mixed or unknown
- Survey type
  - Cross sectional
  - Convenience/screening
  - Case report
  - DHS
- Across surveys, the criteria for selecting samples to test for *pfhrp2/3* deletions varies. Percentage of samples tested with deletions is presented

Global view



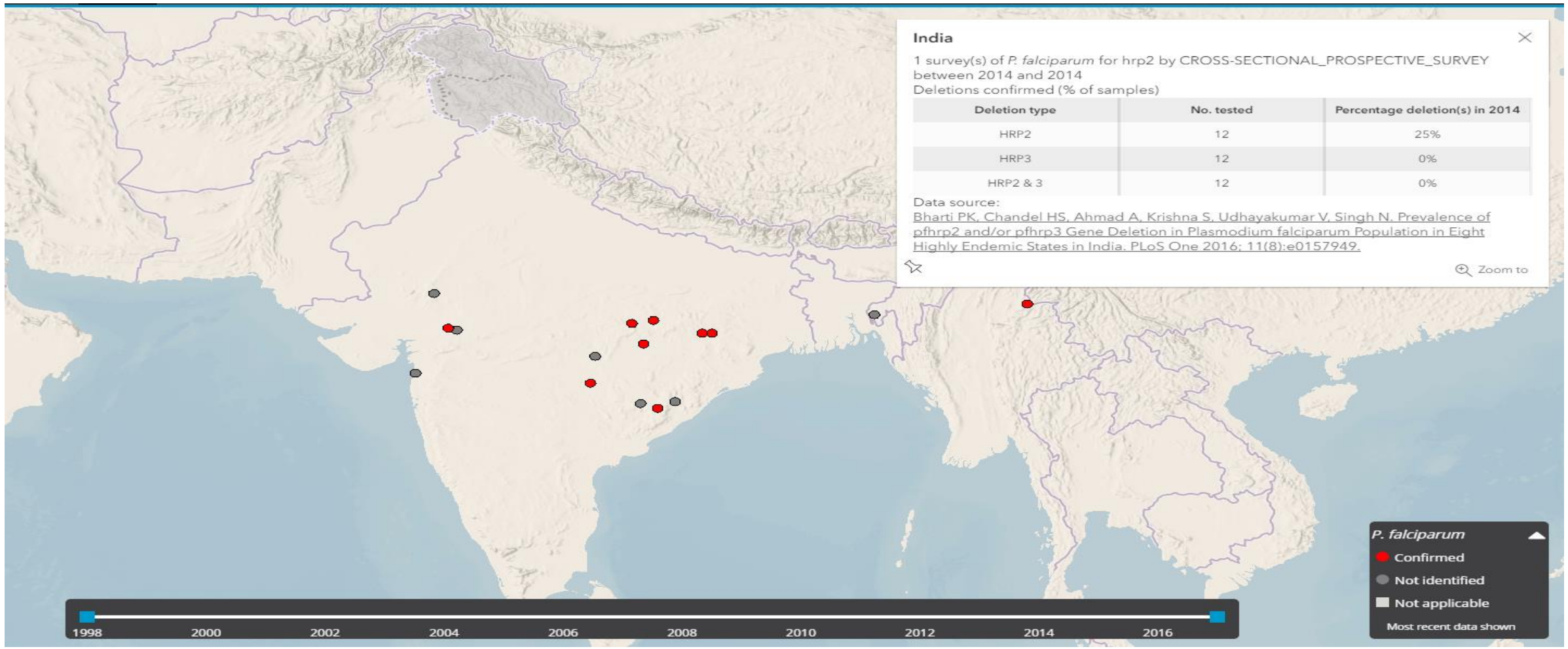
Source: WHO Malaria Threats Map, to be launched October 2017

Specific surveys by country



Source: WHO Malaria Threats Map, to be launched October 2017

Site specific data linked to original source



Source: WHO Malaria Threats Map, to be launched October 2017

Priority areas of work

Discussion point #3

- mapping the distribution and frequency of *pfhrp2/3* deletion mutants with harmonized protocols and creation of a registry of *pfhrp2/3* prevalence surveys;
- building and funding an international network of laboratories to perform the complex molecular confirmation required for mapping;
- supporting countries in the selection and procurement of new RDTs when a change of testing is warranted and regularly updating policy;
- advising commercial manufacturers of the priorities for new tests, including development of target product profiles and providing the best available market forecasts;
- adapting the WHO malaria RDT product testing programme, which constitutes the laboratory evaluation component of WHO prequalification, to ensure proper validation of tests for the detection of *pfhrp2* deletion mutants as part of their intended use;
- working with donor agencies and research institutes to devise a funding plan to support (i) the interim costs for prevalence surveys and the necessary molecular testing (ii) the search for improved diagnostic targets and high-affinity reagent and (iii) short-term operational and technical research to address the problem with clear timelines and financing needs
- strengthening coordination among policy-makers, NMCPs and their implementing partners, molecular testing laboratories, diagnostic industry representatives, donors and technical agencies to maximize efficiency in tracking and responding to this novel situation.

Conclusions

- The full extent of the threat posed by *pfhrp2* deletions is not yet known and the alternative RDT options ie. pf-pLDH RDTs are extremely limited and currently have inferior performance to HRP2 RDTs for *P. falciparum* detection
- Information on prevalence in much of the world is spotty
- Must balance risk of missed cases of falciparum malaria due to *pfhrp*2/3 deleted strains against the equally real risk of missing cases by changing to a less sensitive RDT and the longer term risk of eroding confidence in antigen-based confirmatory testing for malaria

Achieving these goals within the time frame necessary to satisfy the needs of National Malaria Control Programs and the populations they serve will require a focused, staffed, and budgeted effort, and a mechanism for programme management

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Acknowledgements

Mark Perkins, Global Health Diagnostics

Tom Eisele , Tulane University

Joe Keating, Tulane University

Survey protocol

Box 2. Standardized protocol for assessing national prevalence of *pfhrp2/3* deletion mutants among patients with falciparum malaria

Subjects: Symptomatic patients with suspected falciparum malaria among those seen at 10 health facilities in selected provinces

Screening method: A high-quality RDT for detecting HRP2 and either microscopy or a second RDT for detecting PfpLDH

Selection criteria: All falciparum patients with uncomplicated malaria, for whom a suspected false-negative HRP2 RDT was found and who agree to participate

Study method: Collect, label and dry a minimum of two fingerprick blood spots for molecular analysis. Conduct PCR testing of a dried blood spot for (i) species confirmation, (ii) level of parasitaemia, (iii) amplifiable DNA and (iv) the presence of genes that encode HRP2 and HRP3.

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World Health Organization

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Global **Malaria** Programme

False-negative RDT results and implications of new reports of *P. falciparum histidine-rich protein 2/3* gene deletions

MAY 2016 (REV. SEPTEMBER 2017) INFORMATION NOTE

TARGET READERSHIP

National malaria control programme managers and their implementing partners, procurement agencies, national regulatory authorities for in-vitro diagnostics and manufacturers of malaria rapid diagnostic tests (RDTs).

PURPOSE

To provide updated information on the implications of reports of *histidine-rich protein 2/3* (*pfhrp2/pfhrp3*) gene deletions in *Plasmodium falciparum* parasites for case management and to advise on procedures for investigating suspected false-negative RDT results.

BACKGROUND

Most of the currently available commercial RDT kits work by detecting a specific protein expressed only by *P. falciparum*, called HRP2, in the blood of people infected with falciparum malaria. The antibodies on the test strip recognize the PfHRP2 antigen but may cross-react with protein expressed by another member of the HRP gene family, *pfhrp3*, because of the strong similarity of the amino acid sequence. The general preference for PfHRP2-based RDTs in procurement is due largely to the finding in some studies that they are more sensitive and heat-stable than RDTs that detect other malaria antigens, such as plasmodium lactate dehydrogenase (pLDH) – pan (all species) or *P. falciparum*-specific – or aldolase.

World Health Organization

WHO/HTM/GMP/2017.18

In certain situations, HRP2-detecting tests are less sensitive, particularly for parasites that express little or no target antigen, resulting in a false-negative result. In 2010, Gamboa et al.[1] reported the first confirmed identification of *P. falciparum* parasites with *pfhrp2/pfhrp3* gene deletions, which expressed neither PfHRP2 or PfHRP3, in the Amazon River basin in Peru. Subsequent retrospective analyses[2] at different sites in the Loreto region of the Peruvian Amazon showed a statistically significant increase in the number (and percentage) of parasites with gene deletions between specimens collected in 1998–2001 (20.7%) and in 2003–2005 (40.6%). The prevalence of parasites with *pfhrp2/pfhrp3* gene deletions varies, however, from locality to locality. Publications followed from other countries, such as India, Mali and Senegal, but with much lower prevalence estimates, and some studies were based on a flawed design and/or had incomplete analyses.[3] There have been no reports of parasites failing to express pLDH or aldolase, the other antigens targeted by malaria RDTs, as these targets are essential enzymes for parasite metabolism and survival.

In light of recent reports of HRP2 deletions in parasites in several African countries, including the Democratic Republic of the Congo,[4] Eritrea,[5] Ghana,[6] Kenya,[7] Rwanda[8] and India,[9] WHO is providing guidance to RDT manufacturers, procurers, implementers and users on confirming (or excluding) new geographical foci of parasites with deleted *pfhrp2/pfhrp3* and on investigating other causes of suspected false-negative RDT results.

The guidance is updated to include the conclusions and recommendations of a WHO technical consultation on *pfhrp2/3* gene deletions in July 2016 and the results of round 7 of WHO malaria RDT product testing (http://www.who.int/malaria/publications/atoz/978924151268/en/).

POTENTIAL CAUSES AND INVESTIGATIONS INTO SUSPECTED FALSE-NEGATIVE RDT RESULTS

In most settings, genetic mutations like deletion of *pfhrp2/pfhrp3* in parasites are not likely to be the main cause of false-negative results in RDTs, and more studies are required to determine the true prevalence of these mutations. False-negative RDT results are more likely to be due to the procurement and use of poor-quality RDTs or use of the wrong comparator for the diagnostic test, such as poor-quality microscopy for cross-checking negative RDT results.[10] Poor transport and storage conditions for RDTs, with sustained exposure to high temperature, can affect their diagnostic performance. More rarely, operator errors during performance and/or interpretation of RDT results can result in false-negative results. Table 1 lists the product, operator, supply chain, host and parasite factors that can lead to false-negative RDT results and suggested means to investigate such cases. Many of the potential causes of false-negative results can be prevented or minimized by procuring good-quality RDTs, by improving the quality control of procured RDTs (lot verification) and by good training of users.

TABLE 1.
Causes of false-negative RDT results and investigative actions

CLASSIFICATION	CAUSE OF FALSE-NEGATIVE RDT RESULT	SUGGESTED ACTIONS
Operator factors	Operator error in preparing the RDT, performing the test or interpreting the result	Verify whether RDTs are used by untrained staff; assess RDT competence on site.
Use of an imperfect "gold standard" as a comparator	Thick or thin films from a patient with a negative RDT result are incorrectly interpreted as "positive" by microscopy.	Verify microscopy procedures and interpretation by a qualified microscopist.
Product design or quality	Poor sensitivity of an RDT due to poor specificity, affinity or insufficient quantity of antibodies. Poor packaging can result in exposure to humidity, which will rapidly degrade RDTs.	Inspect the instructions for errors; inspect the integrity of the packaging, including the colour indicator desiccant for evidence of moisture. Cross-check suspected false-negative RDT results against microscopy performed by two qualified microscopists or, if microscopy is not available, against a high-quality non-HRP2-detecting RDT; retrieve RDTs from affected areas, and send for lot testing to WHO- or FIND-recognized laboratories.*
	Poor visibility of test bands due to strong background colour on the test	Assess RDT performance and training on site; if the strong background colour persists, notify the manufacturer.
	Incorrect instructions for use	Review the instructions for use for accuracy.
Transport or storage conditions	Antibody degradation due to poor resistance to heat or incorrect transport or storage, e.g. exposure to high temperatures, freeze-thawing	Inspect temperature monitoring of RDT transport and storage chain to determine whether temperatures exceed maximum storage temperature, typically 30 °C or 40 °C or < 2 °C. If temperatures are not within those in the manufacturers instructions, send the RDTs to the WHO-FIND lot testing laboratory.* Train health workers to respect storage conditions, and improve storage places (e.g. add fans).
Parasite factors	Parasites lack or express low levels of the target antigen, i.e. HRP2	Patient samples are negative on an HRP2 test line of at least two quality-assured malaria RDTs **and** positive on the pan- or pf-pLDH test line of a combination RDT **and** the sample is confirmed to be positive microscopically for *P. falciparum* by two qualified microscopists. If these conditions are met, place fresh blood samples or dried blood spots (50-60 µL) on Whatman® 3MM filter paper or other collection cards, in frozen storage (-20 °C) until shipment for PCR and *pfhrp2/pfhrp3* gene analysis.
	Variation in the amino acid sequence of the epitope targeted by the monoclonal antibody	Repeat test with an RDT of a different brand or different manufacturer that targets the same antigen or an RDT that targets a different antigen, e.g. pan-pLDH or Pf-pLDH. Manufacturers may use monoclonal antibodies that target different epitopes of the same antigen.
Host parasite density	Very low parasite density or target antigen concentration	Perform high-quality microscopy, and record the parasite count; if high-quality microscopy is not available, repeat the RDT if symptoms persist.
	Very high parasite load (severe malaria) causing prozone effect (hyperparasitaemia and antigen overload)	Repeat testing with a 10 × and if needed a subsequent 50 × dilution of the sample, with dilutions in 0.9% NaCl **

Note:

* Information about lot testing can be found here: http://www.who.int/malaria/areas/diagnosis/rapid-diagnostic-tests/evaluation-lot-testing/en/

** Gillet et al. Prozone in malaria rapid diagnostics tests: how many cases are missed? Malar J 2011;10:166. https://doi.org/10.1186/1475-2875-10-166

Thousands of febrile children with negative RDT results have been followed up in several studies,[11,12] which showed no malaria-related deaths or hospitalizations. In many endemic areas, malaria prevalence rates have fallen to low levels, and the majority of accurately performed RDTs give negative results. Treatment of individuals with negative RDT results promotes drug resistance, wastes resources and can delay diagnosis of non-malaria causes of fever. In some circumstances, however, false-negative RDT results should be suspected, and an investigation should be carried out to determine the quality of the RDTs, the competence of the operator and/or the presence of *hrp2/hrp3* deletions.

When should false-negative RDT results be suspected for individual patients?

- A symptomatic patient with an initially negative RDT who presents with persistent signs or symptoms of malaria and repeated negative RDT results but a positive blood film interpreted by a qualified microscopist or a positive result with a different quality-assured RDT that targets a different *falciparum-specific malaria antigen (e.g. pf-pLDH) or is of the same brand but from a different lot.*
- A patient with signs or symptoms of malaria with a negative HRP2-based RDT result, who recently visited an area that is known to have a high prevalence of *pfhrp2/hrp3*-deleted parasites, such as Eritrea and Peru.

When should false-negative RDT results be suspected for a population living in a certain geographical area?

- Discordance between RDT and microscopy results, with ≥ 10–15% higher positivity rates by microscopy and routine quality control by cross-checking or when both tests are performed on the same individuals (e.g. during surveys).
- The national malaria control programme and/or the RDT manufacturer receives multiple formal complaints or anecdotal evidence of RDTs returning inaccurate results.

WHEN AND HOW SHOULD FALSE-NEGATIVE HRP2-DETECTING RDT RESULTS DUE TO SUSPECTED PFHRP2 DELETION BE INVESTIGATED?[13]

A *pfhrp2* deletion should be strongly suspected if a patient sample gives negative results on an HRP2 test line of at least two quality-assured malaria RDTs[14] **and** positive on the pan- or pf-pLDH test line when a combination test is used, **and** the sample is confirmed microscopically to be positive for *P. falciparum* by two qualified microscopists.

If a *pfhrp2* gene deletion is suspected and the conditions described above are met:

- Immediately inform the National Malaria Control Programme and WHO;
- Archive the labelled RDTs and slides in a dry, clean area;

- Collect at least 50 µL of blood (about one drop) onto filter paper (e.g. Whatman® 3MM) or appropriate collection cards optimized for DNA analysis;[15] air-dry filter paper or cards overnight in a clean environment, sealed in air-tight plastic bags with desiccant.[16]

- Confirm the presence of *P. falciparum* infection by PCR analysis according to established protocols and with appropriate standards and quality control measures.

- If PCR is positive, confirm *pfhrp2/hrp3* gene deletion by PCR and antigen analysis at laboratories experienced in this kind of assay. WHO/GMP can facilitate linkages with such laboratories and provide further guidance. Contact: cunninghamj@who.int, with the subject line: "Laboratory support for investigations into suspected pfhrp2/3 gene deletions".

IMPLICATIONS OF *PFHRP2/HRP3* MUTATIONS OR DELETIONS FOR PROGRAMMES

Attributing false-negative results to *pfhrp2/pfhrp3* deletion has significant implications for public health. Alternative RDTs will have to be procured, and case management decisions will have to be revised, with re-training in algorithms and RDTs. Therefore, all investigations must be carried out systematically and accurately.

Following confirmation of *pfhrp2* deletions in initial case investigations, blood collection surveys should be made of confirmed *P. falciparum* cases in the specific geographical region to determine the prevalence of parasites carrying gene deletions. Representative samples are required to establish reliable estimates of the prevalence of these parasites. In September 2017, WHO will publish a standard survey protocol for determining whether the number of *pfhrp2* deletions that cause negative HRP2 RDT results among symptomatic patients with confirmed *P. falciparum* malaria has reached a threshold for a change in diagnostic strategy. This protocol will include a sampling tool, report form and data entry templates.

ALTERNATIVES TO HRP2-BASED RDTS

If *pfhrp2* deletions are found to be prevalent among symptomatic individuals (lower 95% confidence interval is > 5%), as, e.g. in Eritrea and several countries in South America (Brazil, Colombia, Peru), country programmes will have to switch to RDTs that do not rely exclusively on HRP2 for detecting *P. falciparum*. A threshold of 5% was selected because it somewhere around this point that the proportion of cases missed by HRP2 RDTs due to non-hrp2 expression may be greater than the proportion of cases that would be missed by less-sensitive pLDH-based RDTs. A recommendation to switch is further informed by mathematical models that show whether parasites lacking *pfhrp2* genes will spread under HRP2-only RDT pressure; a switch may also be decided because of the complexity of procuring and training in use of multiple RDTs. Any change should be applied nationwide, although roll-out might be prioritized on the basis of the prevalence of *pfhrp2* deletions.

Table 2 illustrates the performance of RDTs evaluated in the WHO malaria RDT product testing programme[18] for diagnosis of *P. falciparum* malaria by detection of non-HRP2 antigens, namely *Plasmodium* lactate dehydrogenase (pLDH), pan (pan-pLDH; all species) and *P. falciparum*-specific (pf-pLDH). The products are coded by colour on the basis of whether they meet the recommended procurement critieria for detection of *P. falciparum* in test lines targeting HRP2, pf or pan-pLDH or both. In areas with both *pfhrp2*-deleted parasites and non-*pfhrp2*-deleted parasites, a combination RDT should meet minimum performance criteria for *P. falciparum* detection based on HRP2 and separately based on pf-pLDH. However, for surveys, owing to the scarcity of RDTs that meet performance criteria for Pf detection based on pf-pLDH, RDTs that detect pf-pLDH can be used if their panel detection score is > 90 at 2000 parasites/μL and their false-positive and invalid rates are < 2%. Further details of e.g. heat stability, false-positive results for non-*P. falciparum* infections and test band intensity should be consulted in product testing reports.

The current RDT product testing programme is based on *P. falciparum* culture and clinical samples that express HRP2. This is problematic for assessing the performance of products in which HRP2 and pf-pLDH are on the same test line (products 1, 4 and 6 in Table 2). These tests cannot be evaluated for both antigens; this can be done only when the two antigens are shown on separate test lines. To address this problem, WHO and collaborators are establishing a panel of wild-type and cultured *pfhrp2*-deleted parasites that include both *pfhrp3*-positive and *pfhrp3*-negative combinations, which will be tested in round 8 of WHO malaria RDT product testing. Thus, new data will become available on the performance of these dual antigen test lines as well as on other combination tests that have separate HRP2 and non-HRP2 Pf target antigens.

Where microscopy is available, services should be strengthened to ensure that parasitological confirmation of malaria continues during the transition to new RDTs and for investigations of new foci of suspected *pfhrp2*/*pfhrp3*-deleted parasites.

TABLE 2

Non-HRP2-based RDTs for detecting *P. falciparum* malaria evaluated in WHO malaria RDT product testing rounds 1–7

	PRODUCT	PRODUCT CODE	MANU-FACTURER	PANEL DETECTION SCORE [a]										ROUND	MEETS WHO PROCUREMENT CRITERIA
				200 parasites/µl					2000 parasites/µl						
				Pf samples [c]				Pv samples[d]	Pf samples[c]				Pv samples[d]		
				HRP2/pf-pLDH (dual antigen single test line)	HRP2 test line	pf-pLDH test line	pan-pLDH only test		HRP2/pf-pLDH (dual antigen single test line)	HRP2 test line	pf-pLDH test line	pan-pLDH only test			
	Pf only														
1	CareStart™ Malaria Pf (HRP2/pLDH) Ag RDTf	RMPM(U)-XXX7X	Access Bio, Inc.	91.0				NA	99.0	NA	NA	NA	NA	6	Yes , but only for HRP2 based Pf diagnosis; unknown performance of pf-pLDH alone for detection of *pfhrp2* deleted parasites
2	CareStart™ Malaria Pf (HRP2/pLDH) Ag Combo 3-Line [e]	RMSM-05071	Access Bio, Inc.	NA	94.0	38	NA	NA	NA	99.0	92.0	NA	NA	7	Yes, but only for HRP2 based detection of Pf or as screening test in surveys for *pfhrp2* deleted parasites
3	SD Bioline Malaria Ag P.f (HRP2/pLDH) [e, f]	05FK90	Standard Diagnostics, Inc.	NA	87.0	52.0	NA	NA	NA	100.0	97.0	NA	NA	6	Yes, but only for HRP2 based detection of Pf or as screening test in surveys for *pfhrp2* deleted parasites
4	SD BIOLINE Malaria Ag P.f. (HRP2/pLDH) 2 Lines	05FK130-40-0	Standard Diagnostics, Inc.	90.0	NA	NA	NA	NA	100.0	NA	NA	NA	NA	7	Yes, but only for HRP2 based Pf diagnosis; unknown performance of pf-pLDH alone for *pfhrp2* deleted parasites
	Pf and Pan														
5	CareStart™ MALARIA Pf/PAN (pLDH) Ag RDT	RMLM-05071	Access Bio, Inc.	NA	NA	73.0	NA	0.0	NA	NA	100.0	NA	71.4	7	No for Pf or Pv detection
6	CareStart™ Malaria Screen RDT	RMAM-05071	Access Bio, Inc.	93.0	NA	NA	NA	94.3	99.0	NA	NA	NA	97.1	7	Yes, but only for HRP2 based Pf diagnosis; unknown performance of pf-pLDH alone for detection *pfhrp2* deleted parasites

	PRODUCT	PRODUCT CODE	MANU-FACTURER	PANEL DETECTION SCORE [a]										ROUND	MEETS WHO PROCUREMENT CRITERIA
				200 parasites/µl					2000 parasites/µl						
				Pf samples [c]				Pv samples[d]	Pf samples[c]				Pv samples[d]		
				HRP2/pf-pLDH (dual antigen single test line)	HRP2 test line	pf-pLDH test line	pan-pLDH only test		HRP2/pf-pLDH (dual antigen single test line)	HRP2 test line	pf-pLDH test line	pan-pLDH only test			
	Pf and Pv														
7	BIOCREDIT Malaria Ag Pf/Pv (pLDH/pLDH)	C60RHA25	RapiGEN Inc.	NA	NA	75.0	NA	100.0	NA	NA	98.0	NA	100.0	7	Yes
	Pf, Pf and Pv														
8	SD Bioline Malaria Ag P.f/P.f/P.v [e]	05FK120	Standard Diagnostics, Inc.	NA	84.0	36.0	NA	91.4		100.0	98.0		100.0	6	Yes, but only for HRP2 based detection of Pf or as screening test in surveys for *pfhrp2* deleted parasites
	Pan only														
9	CareStart™ Malaria PAN (pLDH) Ag RDT[f]	RMNM(U)-XXX7X	Access Bio, Inc.	NA	NA	NA	84.0	88.6	NA	NA	NA	99.0	97.1	5	Yes
10	Advantage Pan Malaria Card	IR013025	J. Mitra & Co. Pvt. Ltd.	NA	NA	NA	77.0	100.0	NA	NA	NA	98.0	100.0	5	Yes

UK, unknown
NA, not applicable
Pf, *Plasmodium falciparum*
Pv, *Plasmodium vivax*
pan, *Plasmodium* species
Pvom, *Plasmodium vivax, ovale* and *malariae*

Meets procurement criteria for case management

Does not meet procurement criteria for case management

Meets criteria only for use in surveys for pfhrp2 deletions

PERFORMANCE MEASURE	RECOMMENDED WHO PROCUREMENT CRITERIA
Panel detection score for Pf and Pv 200p/µL samples	≥ 75%
False-positive rate against clean-negatives	<10%
Invalid rate	<5% of tests conducted

a According to methods of WHO malaria RDT product testing a sample is considered detected only if all RDTs from both lots read by the first technician, at minimum specified reading time, are positive

b The total number of times a positive result for malaria was generated when it should not have been

c Round 1, n=79; Round 2, n=100; Round 3, n=99; Round 4, n=98; Round 5, n=100; Round 6, n=100; Round 7, n=100

d Round 1, n=20; Round 2, n=40; Round 3, n=35; Round 4, n=34; Round 5, n=35; Round 6, n=35; Round 7, n=35

e PDS presented in the table is based on a HRP2 test line and Pf-pLDH test line. The overall result at 200 p/µl based on positive HRP2 or pf-pLDH test line is 88 for 05FK90; 85 for 05FK120 and at 2000p/µl it is 99 for RMSM-05071; 100 for 05FK90; 100 for 05FK120

f Indicates a WHO prequalified product

INTERIM WHO RECOMMENDATIONS

1. Suspected false-negative RDT results should be investigated.

2. *Pfhrp2/3* gene deletions should be suspected and the national malaria control programme and WHO informed when:

   a. a sample from an individual tests negative on the HRP2 line of at least two quality-assured malaria RDTs and positive on the pan- or pf-pLDH test line of a combination RDT and the sample is confirmed by microscopy to be positive for *P. falciparum* by two qualified microscopists;

   b. in a programme, the rates of discordance between the results of RDTs and microscopy are systematically ≥ 10–15%, with higher positivity rates in microscopy, where quality is controlled routinely by cross-checking or both are performed on the same individuals (e.g. during surveys) and/or when the national malaria control programme receives multiple formal complaints or anecdotal evidence of RDTs that give false-negative results for *P. falciparum*.

3. When *hrp2/hrp3* gene deletions have been reported, the baseline prevalence should be determined in the affected country and neighbouring countries. This may require specific surveys or adaptation of planned surveys, such as therapeutic efficacy studies. In September 2017, WHO will publish a standard survey tool for determining the prevalence of false-negative HRP2-based RDT results secondary to *pfhrp2* gene deletions.

4. Confirmatory evidence of deletions should include PCR for *pfhrp3*, in addition to PCR for *pfhrp2*, as HRP3 proteins can show cross-reactivity in HRP2-based RDTs; however, analysis of flanking genes for *pfhrp2* (and *pfhrp3*) and serological confirmation of the absent HRP2 antigen (by ELISA or a second brand of RDT) are optional.

5. A nationwide change to an RDT that includes non-HRP2 target antigens for *P. falciparum* is recommended when the lower 95% confidence interval of the prevalence of symptomatic patients carrying *pfhrp2*-deleted parasites (causing false-negative HRP2 RDT results) is ≥ 5%. If *pfhrp2* deletions are confirmed but the prevalence is < 5%, it is recommended that a change be planned over a longer period, as it is anticipated that *pfhrp2/3*-deleted parasites will persist and spread. A repeated survey after 1–2 years will inform a prioritized roll-out of RDTs that include non-HRP2-based antigens.

   In all other cases, if *pfhrp2* deletions are confirmed in samples from any source, the suggested action is to establish the prevalence of false-negative HRP-based RDT results secondary to *pfhrp2* deletion through representative surveys.

6. Well-preserved archived specimens may be analysed to identify the existence and geographical location of *pfhrp2/pfhrp3*-deleted parasite populations.

7. In the absence of confirmed reports of *pfhrp2/pfhrp3* gene deletions, it is not recommended that new initiatives be taken to find these gene deletions, unless they are prompted by findings described under 2 above.

WHO/GMP RESPONSE

Given the complexity of investigating suspected false-negative RDT results and the risk that parasites that do not express HRP2/HRP3 emerge but are not detected, WHO is conducting the following activities:

- preparing a plan of action for surveillance and response to the emergence and spread of *pfhrp2/3* gene deletions (October 2017);
- preparing standard protocols (and tools) for conducting baseline surveys to determine whether the prevalence of *pfhrp2* deletions that cause negative HRP2 RDT results among symptomatic patients with confirmed *P. falciparum* malaria has reached a threshold for a change in diagnostic strategy (September 2017);
- establishing a panel of *pfhrp2/3*-deleted parasites (cultured and wild-type) for evaluating the performance of non-HRP2 Pf-detecting RDTs;
- establishing a network of laboratories to review and build consensus on laboratory methods for characterizing *pfhrp2/3* gene deletions and linking reference laboratories with field investigators to ensure reliable, accurate reporting of *pfhrp2/*3 gene deletions;
- working with relevant groups to adapt planned surveys to include collection of blood samples for molecular testing for malaria, including analysis of *pfhrp2/pfhrp3*, based on WHO-recommended protocols. Areas affected by these mutations, including neighbouring countries, will be a priority;
- working with research groups that hold collections of recently archived samples to screen for the presence of *pfhrp2/pfhrp3*-deleted parasites;
- rigorously reviewing manuscripts submitted for publications and published reports of *pfhrp2/pfhrp3* deletions to determine the accuracy of claims; and
- encouraging test developers and RDT manufacturers to improve the performance of pLDH-based tests and identify new target antigens.

Endnotes

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10. Kahama-Maro J, D'Acremont V, Mtasiwa D, Genton B, Lengeler C. Low quality of routine microscopy for malaria at different levels of the health system in Dar es Salaam. Malar J 2011;10:332.

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12. Baiden F, Webster J, Tivura M, Delimini R, Berko Y, et al. Accuracy of rapid tests for malaria and treatment outcomes for malaria and non-malaria cases among under-five children in rural Ghana. PLoS ONE 2012;7(4): e34073.

13. Cheng Q, Gatton M, Barnwell J, Chiodini P, McCarthy J, Bell D et al. *Plasmodium falciparum* parasites lacking histidine-rich protein 2 and 3: a review and recommendations for accurate reporting. Malar J. 2014;13:283.

14. Quality-assured RDTs are selected on the basis of WHO-recommended procurement criteria, lot-tested before field deployment by a WHO- or FIND-recognized laboratory and transported and stored in accordance with the manufacturer's recommendations.

15. Stored blood slides and used RDTs could be used as sources of DNA, but they are not ideal.

16. The desiccant in the RDT cassette packaging can be used.

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19. To allow for sampling variation.

Malaria Policy Advisory Committee Meeting
17–19 October 2017, Geneva, Switzerland
Background document for Session 4

World Health Organization

Update on the RTS,S Malaria Vaccine Implementation Programme

October 2017, Geneva, Switzerland

Background

In January 2016, WHO published its first malaria vaccine position paper[1], officially adopting the joint recommendation by the Strategic Advisory Group of Experts (SAGE) on Immunization and the Malaria Policy Advisory Committee (MPAC).

A team at WHO, led jointly by the Directors of the Immunization, Vaccines and Biologicals (IVB) Department and the Global Malaria Programme (GMP), has taken the lead in developing the Malaria Vaccine Implementation Programme (MVIP), which aims to operationalize the recommendation for pilot implementation and rigorous evaluation.

The MVIP will support the subnational introduction of the malaria vaccine in selected areas of three pilot countries (Ghana, Kenya and Malawi) and the rigorous evaluation of the programmatic feasibility of administering the required four doses in children; the vaccine's potential role in reducing childhood deaths; and its safety in the context of routine use.

Update since March 2017

On 24 April 2017, the WHO Regional Office for Africa (AFRO) officially announced Ghana, Kenya and Malawi as the three pilot countries to participate in the MVIP. See press release: http://www.afro.who.int/news/ghana-kenya-and-malawi-take-part-who-malaria-vaccine-pilot-programme

Progress has been made with the three funding agencies, Gavi, the Global Fund and Unitaid, to formalize the terms of the agreements expected to provide funding of up to US$ 49.2 million for Phase 1 of the MVIP for the period from July 2017 to December 2020. Finalization of the bilateral agreements is expected in Q3 or 4 2017. Interim funding provided by PATH through a grant from the Bill & Melinda Gates Foundation, together with PATH's existing grants from the Gates Foundation, has so far allowed critical activities to proceed.

GSK is the only supplier of the RTS,S malaria vaccine. The bulk manufacturing site fully dedicated to RTS,S has been idle since 2015, but GSK has recently committed to restarting production in order to meet the needs of the MVIP. This will also lay the foundation for supply capacity in the longer term should the vaccine be recommended for broader use based on the experience from the MVIP. Finalization of the formal collaboration agreement

[1] World Health Organization. Malaria vaccine: WHO position paper – January 2016. *Weekly Epidemiological Record.* 2016;91(4):33–52 (http://www.who.int/wer/2016/wer9104.pdf).

WHO/HTM/GMP/MPAC/2017/16

between WHO, PATH and GSK to define their roles and responsibilities in the MVIP, as well as to quantify the required vaccine supply and outline longer term access provisions, is pending for a meeting in late September.

All pilot countries have initiated the development of vaccine introduction plans, preparatory activities to strengthen pharmacovigilance, and planning for communications activities. First vaccine introduction is currently anticipated for mid-2018.

WHO developed a master protocol for the pilot evaluations with inputs from partners and peer reviewers. The protocol was reviewed by the WHO Research Ethics Review Committee (ERC) on 3 August 2017 and submitted to the European Medicines Agency (EMA) as part of GSK's risk management plan. Feedback from the ERC and EMA reviews will be addressed in a revised version in Q4 2017.

On 18 May 2017, WHO released a Request for Proposals (RFP) to identify research partners to conduct the pilot evaluations in the three pilot countries. Bids were opened on 30 June 2017 and submissions are currently under review by a Proposal Review Committee. Selection in principle of research partners in September 2017 will enable in-depth discussion of their technical and financial proposals in order to proceed, and contracts will be awarded in the final quarter of 2017.

Updates on the MVIP were provided to the AFRO Regional Immunization Technical Advisory Group (RITAG) and the Global Advisory on Vaccine Safety (GACVS) in June 2017. GACVS recommended a set of pharmacovigilance readiness criteria for the three participating countries and will continue to provide advice and support to the pilot countries and to the planned MVIP Data Safety and Monitoring Board.

Preparatory work towards a joint regulatory review of RTS,S by national regulatory authorities from the three pilot countries continued during a meeting on 27–28 June 2017. The joint review will be convened by WHO under the auspices of the African Vaccine Regulatory Forum (AVAREF) and is expected to focus on the evaluation of the EMA article 58 opinion, as well as on other relevant information provided by GSK. This review will provide the basis for the special authorization of the vaccine for use in the MVIP in each of the three countries.

A Programme Advisory Group composed of independent external experts is being convened on 4–5 October 2017 to provide technical advice and recommendations to the MVIP Leadership (which includes the Assistant Director Generals of the WHO clusters hosting GMP and IVB, the Regional Director of WHO AFRO, and the Directors of IVB, GMP and AFRO) and to the Programme Coordination Group.

Contact

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Malaria Vaccine Implementation Programme Update

MPAC

17 Oct, 2017

Mary J. Hamel, MVIP Lead, IVR

David Schellenberg, MVIP focal Point, GMP

World Health Organization

RTS,S Malaria Vaccine Implementation Programme (MVIP) Background

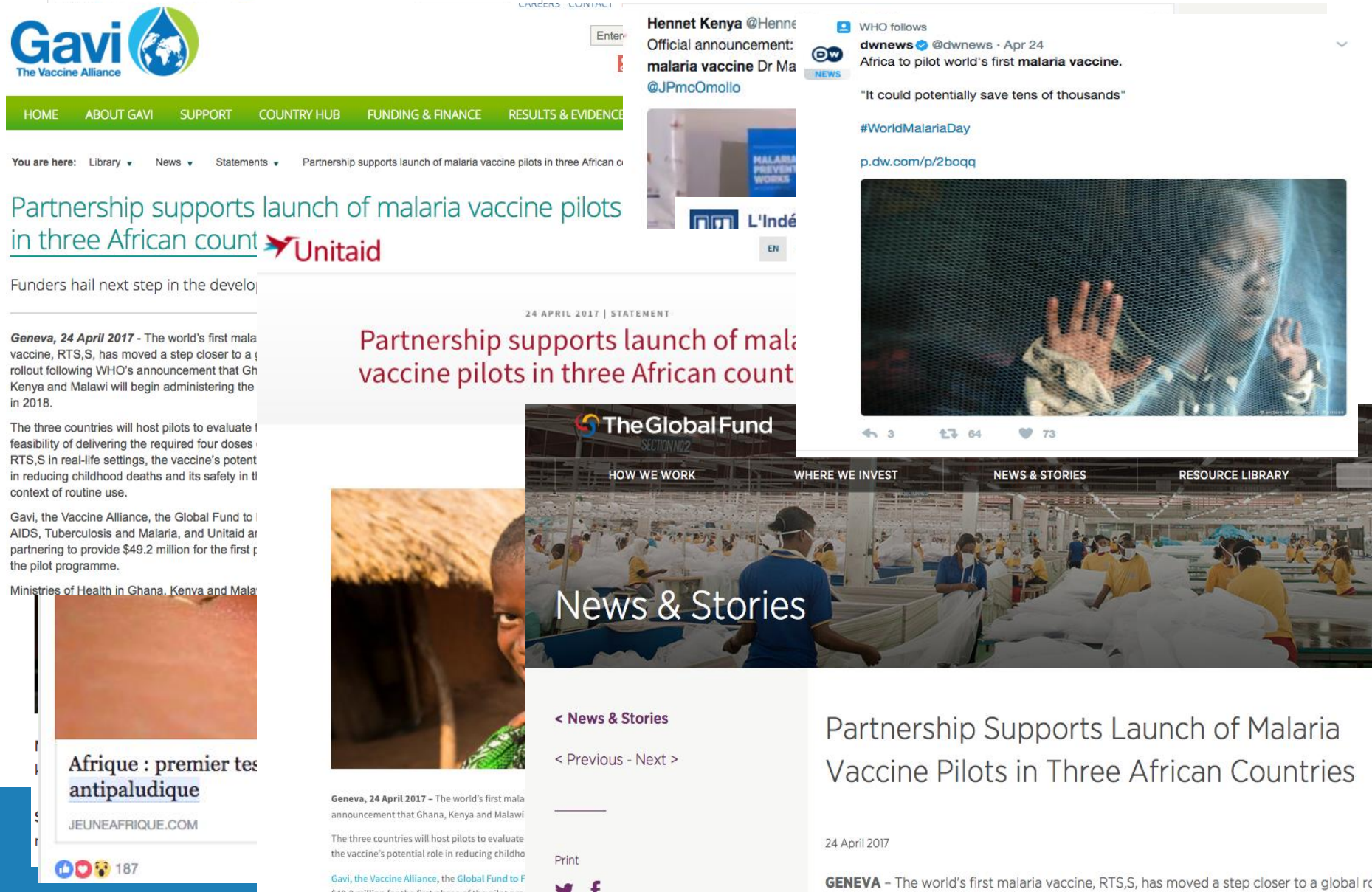
- RTS,S - a Phase 3 trial in >15,000 children
  - Over 4yr in children 5-17 months of age, receiving 4 doses:
    - 39% reduction in clinical malaria,
    - 31% reduction in severe malaria
    - 63% reduction severe malaria anaemia
    - 29% reduction blood transfusions
  - Potential for high impact in moderate to high transmission areas
  - Provision of 4th dose essential to retain impact on severe malaria
  - Potential safety signals: meningitis, algorithmically defined cerebral malaria
  - *Post hoc* analysis gender difference in all cause mortality
- Positive scientific opinion from the European Medicines Agency
  - "Acceptable safety profile", "benefits outweigh risks"

RTS,S Malaria Vaccine Implementation Programme (MVIP) Background

- SAGE and MPAC considered programmatic context, recommended implementation in phased pilots in selected countries, through the EPI programme as new vaccine introduction, using routine systems
- Accompanied by rigorous evaluation to determine the public health role of RTS,S vaccine
  - **Feasibility**, particularly administering 4 doses with additional contacts
  - **Impact** on severe malaria and mortality (including by gender)
  - Consolidate **safety** profile, with emphasis on meningitis and cerebral malaria
- 3 countries to implement RTS,S vaccine in selected subnational areas: **Kenya, Malawi, Ghana**

Country Selection Announcement

24 April 2017 in Nairobi (WMD and AVW)



MVIP Progress to Date

- Tripartite agreement between PATH, GSK, WHO
  - Outlines roles and responsibilities
  - Pending finalization following meeting 25 September
  - GSK has committed to re-start production of RTS,S/AS01 to support the pilot
- Funding
  - Joint funding agreement finalized (Gavi, Global Fund, UNITAID)
  - Finalizing bilateral agreements
  - Funds available when collaborative tripartite agreement signed
  - Bridging funds from PATH supporting programme in interim

MVIP Progress to Date

- Regulatory
  - Meeting of NRAs convened under African Vaccine Regulatory Forum (AVAREF), June 2017
  - Joint regulatory review of RTS,S vaccine planned for Q4 2017
  - NRAs will seek mechanism to authorize RTS,S for use in pilot areas
- Programme Advisory Group (PAG)
  - Highest advisory group of MVIP
  - Will review reports and data from MVIP and will advise WHO on specific technical, scientific, or programmatic questions
  - Includes one MPAC and one SAGE member
  - Inaugural PAG Meeting, Oct 4,5 2017
  - Advised on proposal to develop Framework for policy decision and to convene group of experts to consider meningitis signal
    - Real, chance, systematic bias; If systematic bias, risk to repeat in pilots

MVIP Progress to Date

- Implementation
  - First vaccinations planned for Q2 2018
  - All countries developing new vaccine introduction and pharmacovigilance plans and budgets
  - Presentation to GACVS and RITAG of safety monitoring within the MVIP
    - Recommendation of implementation readiness indicators
- Evaluation
  - Master protocol under review by WHO Ethical Review Committee and through GSK to EMA with risk management plan
  - Master protocol will serve as model for country-specific protocols to be developed by evaluation partners
  - RFP process provisionally identified evaluation partners (EP) for each pilot country
    - Confirmation of EPs will be based on ability of EPs to conduct work within available MVIP budget

Proposal to Develop a Framework for Policy Decision for RTS,S

- MVIP team propose the development of a framework for policy decision on RTS,S that describes how data collected through the implementation pilots will be used to inform policy
- As part of the implementation pilots, data will be collected on feasibility, safety and impact to inform policy decision for RTS,S
- JTEG recommends WHO should also monitor **emerging findings** from pilot implementation[1]
  - Based on those findings, "it would be appropriate for WHO to recommend countrywide introduction if **concerns about safety have been resolved, and if favorable implementation data become available, including high coverage of the fourth dose**"
- Not stated how data will be used
  - What is "high coverage of the fourth dose"?
  - Is demonstration of impact on mortality required for a policy recommendation?

1.JTEG Background Paper on the RTS,S/AS01 Malaria Vaccine, Sep 2015

Potential Usefulness of a Framework for Policy Decision

- Through the development of a framework for policy decision
  - SAGE and MPAC members could discuss and refine ideas on the relative contribution of the collected data (feasibility, safety, impact) to a future policy recommendation
  - Provide clarity on the expected use of the data in anticipation of potential changes in SAGE membership between the time the SAGE/MPAC recommendations were made and the programme end (2022)
  - Funders, potential funders, and manufacturers can refer to the framework for planning purposes
    - Reducing the likelihood of gaps in funding or vaccine availability should the vaccine be recommended for broader use

Not a Go/No Go Decision
Not Binding

- Framework will not provide a Go/No Go decision for policy, and could not be binding
- High-bar, data driven, thresholds defined above which it is very likely the vaccine would be recommended for broader use (e.g. very high (80%) coverage of doses 1 – 4, Safety concerns resolved)
- Should the thresholds not be met, a more nuanced discussion would be required to consider the public health usefulness of the vaccine (e.g. considerations of risk/benefit profile).
- Modellers could assist in identifying vaccine coverage thresholds, using phase 3 data, that predict significant impact on severe malaria or mortality

Considerations within a Framework for Policy Decision

- What criteria, if met, would demonstrate "favorable implementation data" during the course of the pilots and would likely justify broader country-wide implementation?
- Assuming safety concerns have been resolved, would evidence of impact on severe disease result in a recommendation for **broader implementation**, regardless of the absolute coverage of 4th dose?
- What criteria, if met, would likely lead to a recommendation for vaccine use at the end of the pilot implementation programme?
  - Is evidence of impact on mortality required for vaccine recommendation?

Framework for Policy Decision
Next Steps

- The MVIP team proposes to work with the MVIP Programme Advisory Group (PAG), the highest level advisory group to the MVIP
  - Representation from MPAC and SAGE on PAG
- Return to SAGE and MPAC in 2018 to discuss a **draft framework** and whether such a framework, indicators, and thresholds could be useful
- Proposal presented to SAGE on 14 Sept and PAG in October – recommendation from both to move forward
- Is MPAC in agreement with proposal?

Malaria Policy Advisory Committee Meeting
17–19 October 2017, Geneva, Switzerland
Background document for Session 4

Malaria vaccine research and development: Malaria Vaccine Advisory Committee (MALVAC) meeting planning

Initiative for Vaccine Research and Global Malaria Programme
October 2017, Geneva, Switzerland

Background

The WHO Global Malaria Programme (GMP) coordinates WHO's efforts to control and eliminate malaria. Important progress has been realized over the last decade, and the Global Technical Strategy provides a framework for accelerating progress in the reduction of malaria disease and mortality, moving towards elimination and preventing resurgence between 2016 and 2030. Yet, malaria remains responsible for a massive burden of disease and death and, as such, is an important public health priority. The availability of a highly effective malaria vaccine would greatly strengthen the prospects for further, sustained public health gains.

The WHO Initiative for Vaccine Research (IVR) provides guidance in the field of vaccine research and development (R&D) against priority diseases. Public health targets and advice on research avenues are outlined in preferred product characteristics (PPC) and R&D technical roadmap documents. The latest version of the WHO Malaria Vaccine R&D Technical Roadmap was produced in 2013. Since then, the malaria vaccine landscape has evolved in several major ways.

- One product (RTS,S/AS01) has received a favourable scientific opinion from the European Medicines Agency (EMA), having demonstrated moderate levels of protection against uncomplicated and severe malaria in Phase III clinical evaluation. Pilot implementation studies will soon begin in order to further evaluate the effectiveness, safety and feasibility of implementation through routine health systems.

- Other studies will assess the role of the RTS,S/AS01 vaccine when administered before or at the beginning of the malaria season in highly seasonal transmission areas and evaluate the potential of RTS,S/AS01 in South-East Asia. When tested against controlled human malaria infection (CHMI), the administration of a delayed, fractional dose of RTS,S has shown the potential to increase the vaccine's efficacy. The role of dose- and schedule-associated changes to the vaccination regimen will be further evaluated in CHMI and natural exposure studies.

- A new RTS,S-like particle, R21, has been developed. Product characteristics and early experimental evaluation suggest that there are significant differences from RTS,S, warranting further evaluation.

WHO/HTM/GMP/MPAC/2017/17

- New evidence has been generated using live sporozoite immunizations, tested in multiple studies using CHMI or natural exposure.

- The evaluation of other malaria vaccine candidates continues with trials of promising blood-stage and sexual-stage candidate vaccines. Vaccine strategies against *Plasmodium vivax* malaria are being developed and moving into early clinical evaluation.

- Developments in experimental design have seen an increase in the use of CHMI studies and trials involving a second or delayed challenge in order to assess durability of protection. Platforms for CHMI studies have been developed within malaria-endemic countries. A low-dose blood-stage infection model has shown great potential for the measurement of vaccine-induced blood-stage immunity. Progress has been made in the set-up of a human malaria transmission experimental model fit for evaluating the transmission-blocking activity of sexual-stage vaccines.

- Major and rapid changes in malaria epidemiology are being seen at the global level, under constant pressure from malaria control and elimination efforts. Rapid progress is being made in the science of and pathway to malaria elimination.

In this generally evolving context, it is important to reconsider the role of malaria vaccines in the future technical framework for malaria control and elimination.

The Malaria Vaccine Advisory Committee (MALVAC) was established to provide expert input to help WHO articulate its vision and recommendations on malaria vaccine development.

It is timely for MALVAC to reconvene in order to assist WHO in the prioritization of specific malaria vaccine R&D avenues and thus support robust future policy decisions. The state-of-the-art in malaria vaccine development should be reviewed, and priority targets and preferred clinical development pathways should be redefined based on the review of new evidence, consideration of recent activities and changed public health priorities. An updated vision for the role of vaccines in future malaria control and elimination efforts needs to be articulated.

The high-level objectives of the malaria vaccine consultation, MALVAC meeting and the ensuing work are the following:

A WHO vision for malaria vaccines

- Discuss current trends in malaria epidemiology;

- Develop an integrated vision of the role of vaccines in the projected future technical framework for malaria control and elimination;

- Map out the status of malaria vaccine development, recent progress and challenges;

- Discuss potential malaria vaccine use in different epidemiological contexts;

- Update the priority targets as expressed in the malaria vaccine R&D technical roadmap;
- Discuss the vaccines' value propositions: what are the vaccine profiles that can realistically play a role in the foreseeable future (i.e., around 2030)?;
- Develop a vision for *P. vivax* vaccines;
- Consider the potential effect of vaccines on the development and spread of drug resistance.

Research and development avenues

- Provide guidance on the design of early proof-of-concept studies and optimal use of CHMI models, including transmission models;
- Determine the preferred late-stage evaluation strategies for second-generation malaria vaccine candidates and advise on the safety, efficacy and implementability evidence base required for WHO policy decisions;
- Discuss research standards in light of the status of RTS,S/A01;
- Discuss evaluation strategies for vaccines aimed at interrupting transmission.

Malaria Vaccine Advisory Committee

MALVAC

David Schellenberg & Johan Vekemans

Global **Malaria** Programme

Immunization **Vaccines** Biologicals

Malaria Policy Advisory Committee Meeting

Geneva, Switzerland, 17 October 2017

World Health Organization

Background

- The Malaria Vaccine Advisory Committee (MALVAC) was convened to advise WHO on activities related to the development of malaria vaccines
- MALVAC met 8 times between 2008-2013
- Last version of the WHO Malaria Vaccine R&D Technical Roadmap was produced in 2013
- The malaria vaccine landscape continues to evolve
- Timely to re-convene MALVAC

The evolving landscape

- Major changes in malaria epidemiology
- RTS,S/AS01:
  - Pilot implementation to start in 3 African countries mid-2018
  - Study to assess potential in highly seasonal transmission areas
  - Evaluation of potential to help interrupt transmission
  - Fractional dose of RTS,S regimen
- R21 – an RTS,S-like particle – showing promise
- Live sporozoite immunizations
- Other vaccine candidates, including against *P. vivax*

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Health products in the pipeline

Published: May 2017

Candidate health products (e.g. medicines, vaccines and diagnostics) that are currently under development are reported by disease, product name, type, and phase of development. When available, the developer's institution is also indicated.

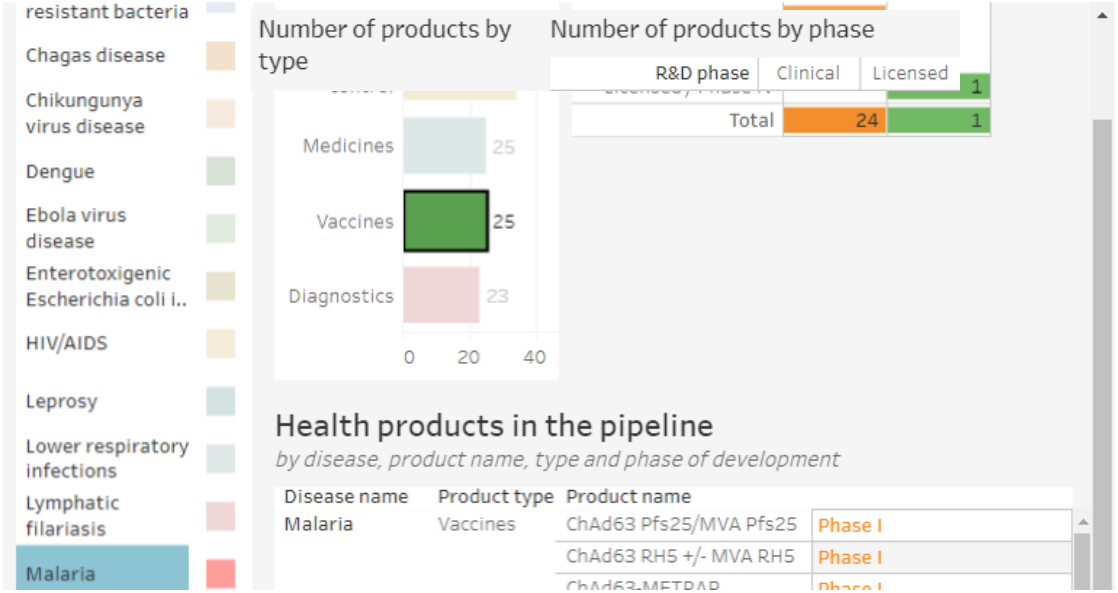
– View version published January 2017

See also:

– Antibacterial products in clinical development for priority pathogens

What you see | Scope and limitations | Data sources

Data visualization 1



www.who.int/research-observatory/monitoring/processes/health_products/en/

Developments in experimental design

- **Controlled human malaria infection** (CHMI) increasingly used
  - Repeat challenge studies to assess duration of protection
  - Development of CHMI platforms in malaria endemic countries
- Low dose **blood stage infection model** with potential to measure vaccine-induced blood stage immunity
- Development of human **malaria transmission experimental model** to evaluate transmission-blocking activity of sexual stage vaccines

Reconvening MALVAC

- Assist WHO in the prioritization of specific malaria vaccine R&D avenues
- Review the state-of-the-art in malaria vaccine development
- Define priority targets and preferred clinical development pathways, mindful of emerging data and changing public health priorities
- Update the vision for the role of vaccines in future malaria control and elimination efforts
- Jointly convened by WHO's Initiative for Vaccine Research (IVR) & GMP

Terms of reference

- Provide advice to WHO on activities related to the development of malaria vaccines
- Facilitate coordination of the international malaria vaccine research and development (R&D) effort, with a special emphasis on public health needs of developing countries
- Facilitate coordination of malaria vaccine development activities in the context of ongoing global malaria control and elimination efforts
- Develop guidance on non-clinical and clinical evaluation of malaria vaccines to support the eventual development of norms and standards around these vaccines
- Identify opportunities for new - or neglected lines of - research

The Committee

- Up to 12 members, appointed by Directors GMP and IVR
  - Expertise in clinical trials of vaccines, public health and epidemiology, vaccine implementation, malariology, malaria control, biostatistics, vaccine safety, immunology and vaccinology, biotechnology.
- Open call for nominations; broad geographic representation, gender balance
- Members act in their personal capacities and should be free of significant conflicts of interest
  - Initially appointed for 2 years, renewable up to two further 2-year terms
  - Chair appointed for 3 years, possibility of one renewal
- Committee may be supplemented by other experts, including those from other WHO advisory groups

Meetings

- As the need arises, no regular fixed interval
- All documents to be treated as confidential - confidentiality agreement upon appointment
- Standard Declaration of Interests procedures
- Observers may be nominated on an as-needed basis by GMP & IVR secretariat prior to each meeting
- A closed session, restricted to committee members and secretariat only, will be scheduled in each meeting
- May establish subcommittees, expert working groups or study groups required to address issues relevant to specific aspects of malaria vaccine development

Malaria Vaccine Consultation

Session 1

- Changing malaria epidemiology - potential roles for malaria vaccines
- Malaria vaccine landscape

Session 2

- Use of human infection models for evaluation of malaria vaccines
  - Mosquito and IV sporozoite inoculation, blood stage controlled infection, models for assessment of transmission blocking, *P vivax* CHMI, use in malaria endemic countries
- Late stage evaluation
  - Lessons from RTS,S
  - Should RTS,S be considered the standard of care in clinical trials testing new malaria vaccines ?
- Evaluation in pregnancy – when and how?

Malaria Vaccine Consultation

Session 3

- Different use scenarios
  - Low, middle and high endemicity settings
  - Contribution to malaria elimination
  - Vaccines to contain drug resistance
  - *P falciparum*, *P vivax* interplay
  - Vaccines against malaria in pregnancy
  - Seasonal vaccination

- Followed by the re-convening of MALVAC
  - Potential for working groups, e.g. to develop Prefered Product Characteristics for different use scenarios

MPAC is invited to comment on the merits of reconvening MALVAC and of organizing a malaria vaccine consultation