Malaria Policy Advisory Committee (MPAC) Meeting

22-24 March 2017
Kofi Anan Room, UNAIDS bldg., World Health Organization, Geneva, Switzerland

PROVISIONAL PROGRAMME*

Thursday, 23 March 2017			
	Session 5	**Open**	**for information**
09:00 – 10:00	Strategic Advisory Group (SAG) on malaria eradication	Dr Marcel Tanner	

(Continue next page)

10:00 – 10:30	*Framework for malaria elimination*	Dr Pedro Alonso	**for guidance**
10:30 – 11:00	*Coffee break*		
	Session 6	**Open**	**for information**
11:00 – 12:30	Report on the ERG on the emergence and spread of multidrug resistant *Plasmodium falciparum* lineages in the Greater Mekong subregion / Presentation	Dr Dyann Wirth	
12:30 – 13:30	*Lunch*		
	Session 7	**Open**	**for guidance**
13:30 – 14:30	Situation update on HRP2/HRP3 gene deletions / Presentation	Dr Jane Cunningham	
14:30 – 15:00	*Mass drug administration for malaria. A practical field manual* (Final version January 2017)	Dr Andrea Bosman	
15:00 – 15:30	*Coffee break*		
	Session 8	**Open**	
15:30 – 16:30	Overview of WHO policy recommendations for malaria vector control interventions	Dr Raman Velayudhan Dr Jan Kolaczinski	**for decision**
16:30 – 17:30	Proposed ERG on submicroscopic malaria infections/ Presentation	Dr Andrea Bosman	**for guidance**
17:30	*End of day*		
Friday, 24 March 2017			
	Session 9	**Open**	**for guidance**
09:00 – 10:00	Malaria Elimination by 2020	Dr Kim Lindblade Dr Gawrie Galappaththy	
10:00 – 11:00	Global call for action to ensure universal access to malaria diagnosis and treatment	Dr Richard Cibulskis Dr Andrea Bosman Dr Jan Kolaczinski	**for guidance**
11:00 – 11:30	Closing remarks	Dr Ren Menghui	
	Session 10	**Closed**	**for decision**
11:30 – 15:00	Finalization of wording of recommendations (including lunch)	Dr Kevin Marsh	
15:00	*End of day*		

** Provisional Programme and may be subject to change*

A framework for malaria elimination

What's new?

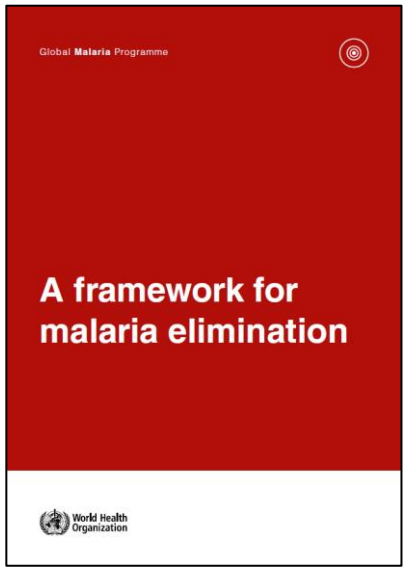
Dr Pedro Alonso, GMP Director

Global **Malaria** Programme

World Health Organization

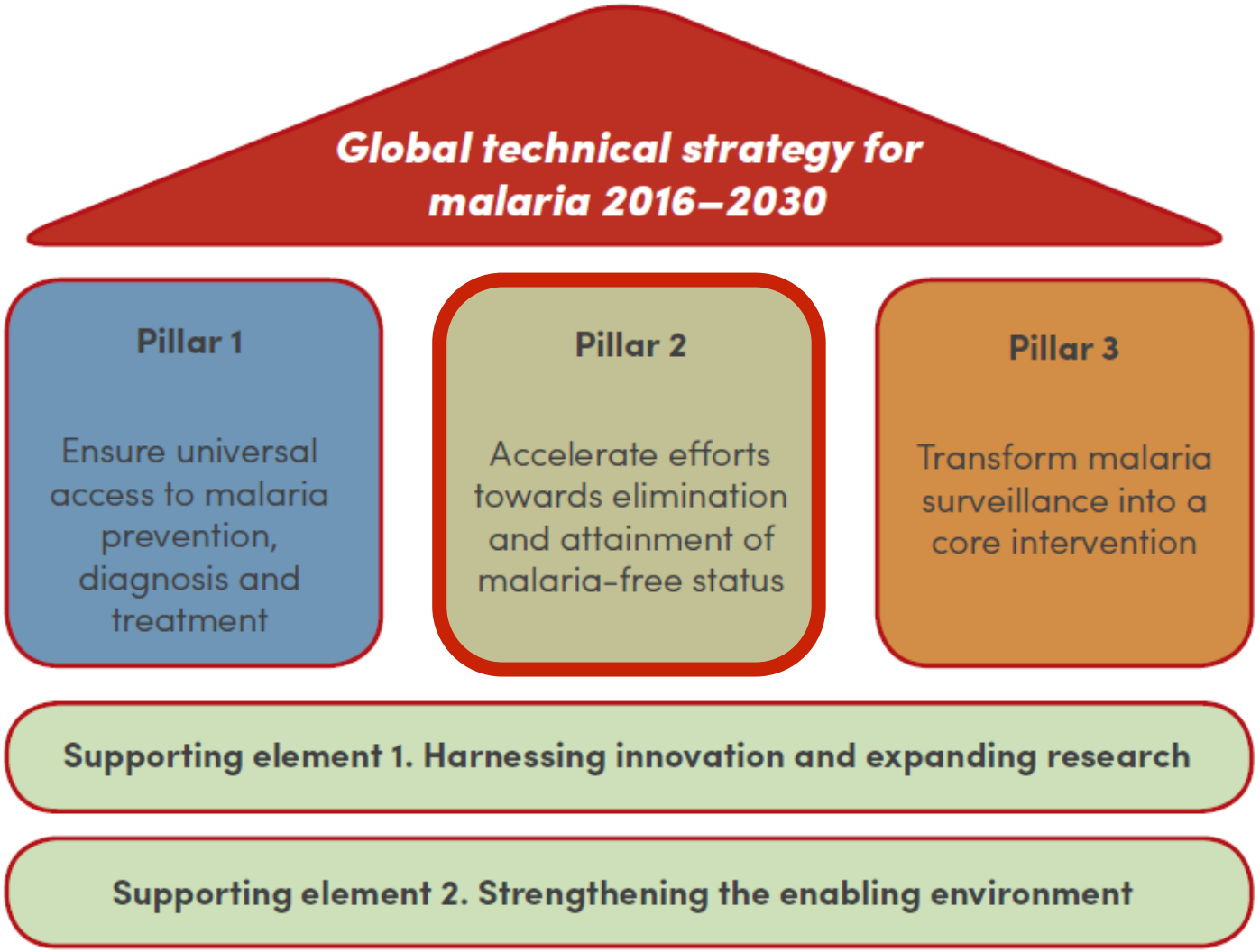
Rationale for new malaria elimination framework

- **The malaria landscape has changed dramatically since 2007**
  - Increased funding for malaria programme activities
  - Large-scale implementation of malaria interventions
  - Impressive reductions in malaria burden
  - Increasing number of countries eliminating or considering elimination of malaria
  - Changes in policy recommendations and available tools
  - Development of new *Global technical strategy for malaria 2016-2030* (3 pillars incl. elimination, 2 supporting elements) – <u>all</u> countries to accelerate towards malaria elimination



Released on March 16, 2017

Malaria elimination reflected in GTS structure, pillars and supporting elements



GTS vision, goals, milestones and targets

Vision – A world free of malaria

Goals	Milestones		Targets
	2020	2025	2030
1. Reduce malaria mortality rates globally compared with 2015	≥40%	≥75%	≥90%
2. Reduce malaria case incidence globally compared with 2015	≥40%	≥75%	≥90%
3. Eliminate malaria from countries in which malaria was transmitted in 2015	At least 10 countries	At least 20 countries	At least 35 countries
4. Prevent re-establishment of malaria in all countries that are malaria-free	Re-establishment prevented	Re-establishment prevented	Re-establishment prevented

Elimination framework, Evidence Review Group

- **13 members with expertise and experience across relevant disciplines:**

  - Dr Rick Steketee, PATH-MACEPA *(ERG Chair)*
  - Dr Majed Al-Zadjali, Department of malaria, MoH, Oman
  - Dr Graham Brown, Nossal Institute for Global Health
  - Dr Tom Burkot, James Cook University
  - Dr Justin Cohen, Clinton Health Access Initiative (CHAI)
  - Dr Mikhail Ejov, independent consultant
  - Dr Rossitza Mintcheva-Kurdova, independent consultant
  - Dr Bruno Moonen, Bill & Melinda Gates Foundation
  - Dr Gao Qi, Jiangsu Institute of Parasitic Diseases
  - Dr Frank Richards, The Carter Center
  - Dr Christophe Rogier, French Military Medical Service
  - Dr Allan Schapira, independent consultant
  - Dr Robert Snow, KEMRI Wellcome Trust Research Programme

Process for development and wide consultation

- Review of the draft guidance
- Definition of clear and detailed outline

- Deep dive into the 2007 manual
- Consensus on the gaps and suggested changes
- Outline of new guidance and each chapter
- Appointment of a focal point to coordinate the process of collecting revised sections and compilation of inputs

Experts to develop working papers, conduct in-depth search or review papers/collect historical experience

Prep. work

1st ERG meeting
New Delhi

2nd ERG meeting
Montreux

July-Aug 2015

Dec 2015

Consultative process

- Discussion at regional meetings
- ITWs with programme managers

Feedback consolidation work

Write, synthesize and rearticulate

(WHO-PATH/MACEPA)

Final review

3rd ERG meeting
Shanghai

March 2016

May 2016

June 2016

Regular teleconferences as needed

Ongoing interactions with GMP coordinators

- Incorporation of inputs from MPAC
- Review of other feedback (all regions and countries)

MPAC presentation

Production & publishing

Sept 2016

March 2017

Launch in Geneva

Global **Malaria** Programme

World Health Organization

What's new? (1)

Key changes from the 2007 field manual:

- Framework addresses **all malaria-endemic countries**
- Programme actions are highlighted across the continuum of transmission, **from high to very low/zero**
- Elimination feasibility replaced by **critical requirements to achieve and maintain elimination**
- Critical role of **information systems and surveillance as an intervention**
- **Planning** for next step has to be done early

What's new? (2)

- **RDTs and light microscopy** recommended for malaria diagnosis
- **Simplified focus classification** (3 vs 7)
- **Updated strategies** for different transmission intensities (e.g. MDA)
- Emphasis on the role / documentation of **verification** of malaria elimination (subnational level) on the way to WHO **certification** of malaria elimination (national level)
- **Simplified process for certification of malaria elimination by WHO**
- Clarified threshold for **re-establishment of transmission**.

Key changes and key concepts

WHO malaria terminology (#1)

- **Malaria elimination**: interruption of local transmission (reduction to zero incidence **of indigenous cases** *[vs locally acquired]*) of a specified malaria parasite **species** in a defined geographic area as a result of deliberate efforts. Continued measures to prevent re-establishment of transmission are required.
- **Certification of malaria elimination** in a country will require that local transmission is interrupted for all **human** malaria parasites.

WHO malaria terminology (#2)

- **<u>Certification of malaria elimination</u>** requires proof that:
  - local malaria transmission has been fully interrupted, **resulting in zero incidence of indigenous cases** for at least the three past consecutive years, and
  - an adequate surveillance and response system for preventing re-establishment of indigenous transmission is fully functional throughout the country.

- A minimum indication of possible **re-establishment of transmission** would be the occurrence of **three or more indigenous malaria cases of the same species per year in the same focus, for three consecutive years.**

Which settings are targeted?

- Framework addresses:
  all epidemiological settings / the whole continuum (high to very low/zero transmission) even if **focus is on low to zero transmission**.

Chapter 1 discusses inclusivity and provides some welcoming of **all** malaria-endemic countries into the realm of ultimately planning for and considering sequential progress towards elimination

From Chapter 2 on

Indicative metrics for transmission intensity

Transmission intensity

High | **Moderate** | **Low** | **Very low** | **Zero**

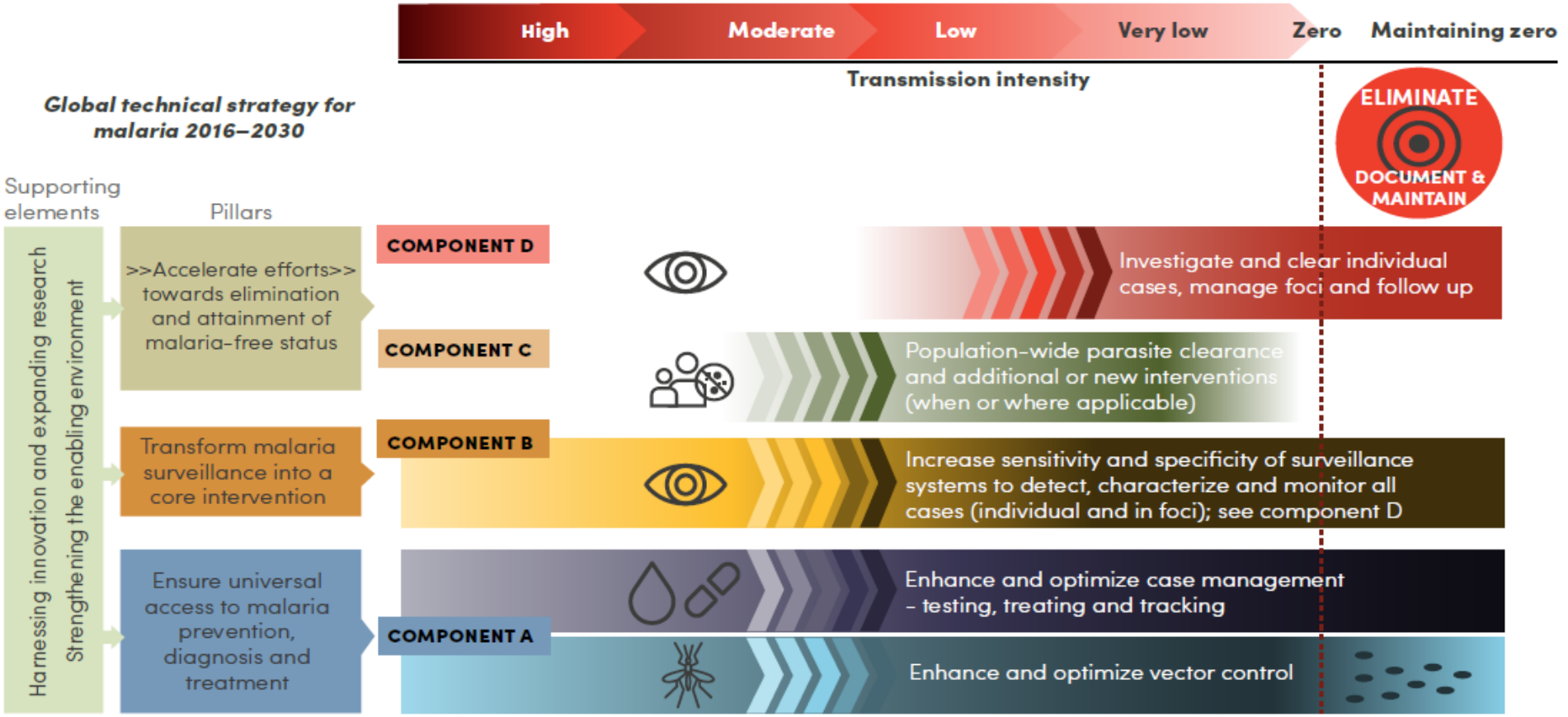
- Annual parasite incidence (API) of ~ **≥ 450 cases** per 1,000
- P. *falciparum* prevalence rate of **≥ 35%**

- API of **250–450 cases** per 1,000
- Prevalence of *P. falciparum/P. vivax* malaria of **10–35%**

- API of **100–250 cases** per 1,000
- Prevalence of *P. falciparum/P. vivax* malaria of **1–10%**

- API of **< 100 cases** per 1,000
- Prevalence of *P. falciparum/P. vivax* malaria **> 0% but < 1%**

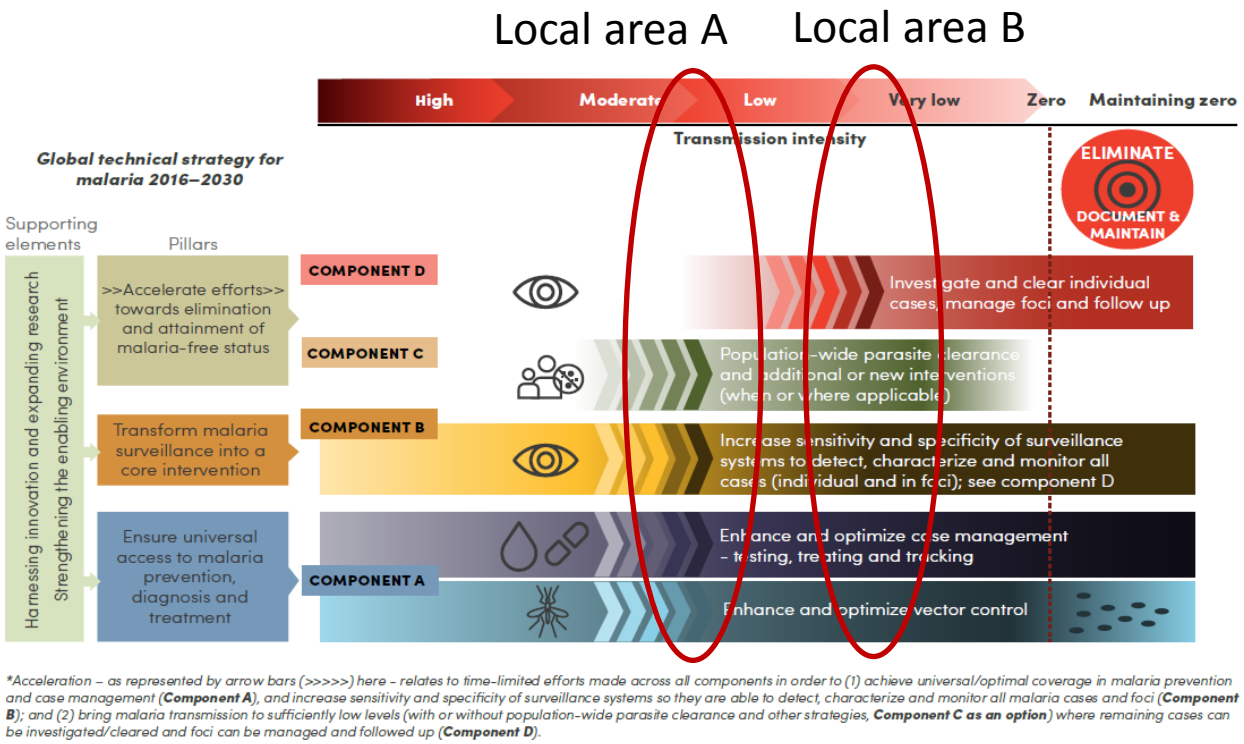
Illustrative intervention package



Acceleration – as represented by arrow bars (>>>>>) here – relates to time-limited efforts made across all components in order to (1) achieve universal/optimal coverage in malaria prevention and case management (Component A**), and increase sensitivity and specificity of surveillance systems so they are able to detect, characterize and monitor all malaria cases and foci (**Component B**); and (2) bring malaria transmission to sufficiently low levels (with or without population-wide parasite clearance and other strategies, **Component C as an option**) where remaining cases can be investigated/cleared and foci can be managed and followed up (**Component D**).*

Illustrative intervention package, continued

- **Need to adapt and tailor interventions to specific geographical areas within the same country – notion of stratification**



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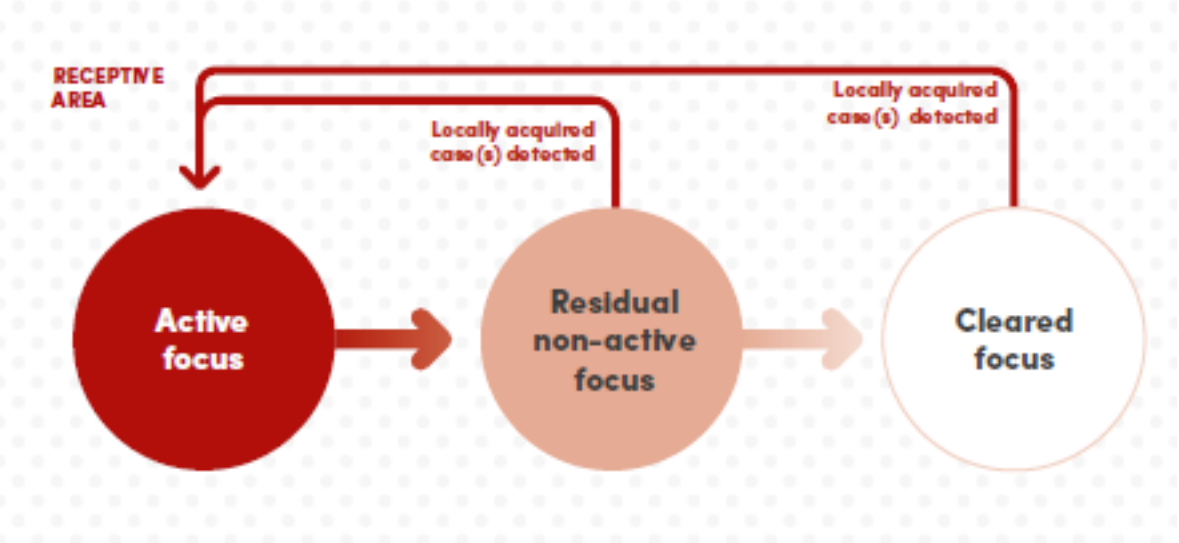
"There is no one-size-fits-all package of interventions suitable for all areas with malaria transmission or transmission risk. Epidemiologic, ecologic, and/or societal features that determine stratification permit national malaria programmes to assign intervention package choices and application methods."

Simplified classification of foci

- **Malaria focus**: A defined and circumscribed area situated in a currently or formerly malarious area that contains the epidemiologic and ecological factors necessary for malaria transmission.

Note: In the 2007 manual, foci were classified as **endemic**, **residual active**, **residual non-active**, **cleared up**, **new potential**, **new active** or **pseudo**.

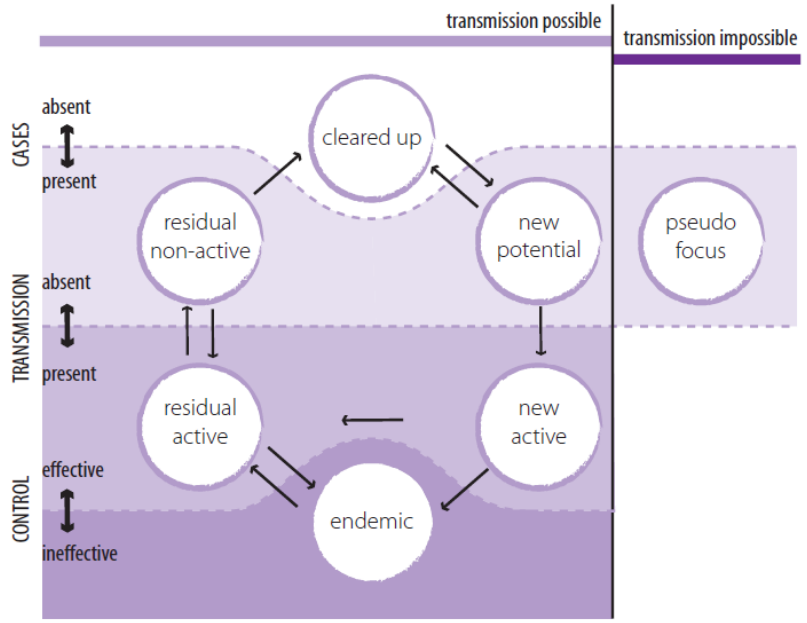
In 2017 Framework, foci are classified as **active, residual non-active** or **cleared**.



Classification of foci, continued

2007 manual

Figure 4. Transition of functional status of a malaria focus depending on the situation



[a] Adapted from *Guidelines on the elimination of residual foci of malaria transmission* (2).

2017 Classification of foci

Table 3. Types of malaria foci with operational criteria and recommended minimum standards of response

Type of focus	Definition	Operational criteria
Active	A focus with ongoing transmission	Locally acquired case(s) have been detected within the current calendar year.
Residual non-active	Transmission interrupted recently (1–3 years ago)	The last locally acquired case was detected in the previous calendar year or up to 3 years earlier.
Cleared	A focus with no local transmission for more than 3 years	There has been no locally acquired case for more than 3 years, and only imported or/and relapsing or/and recrudescent cases or/and induced cases may occur during the current calendar year.

Updated strategies are recommended

New guidance since 2007

- Universal coverage of vector control interventions for at-risk populations
- Diagnostic testing: Rapid diagnostic tests OR microscopy
- Mass drug administration is now a recognized strategy that can be considered for accelerating the time to elimination; it may be needed only in some settings
- Revised malaria treatment guidelines
- *Plasmodium vivax* strategy

Diagnostic testing in elimination settings

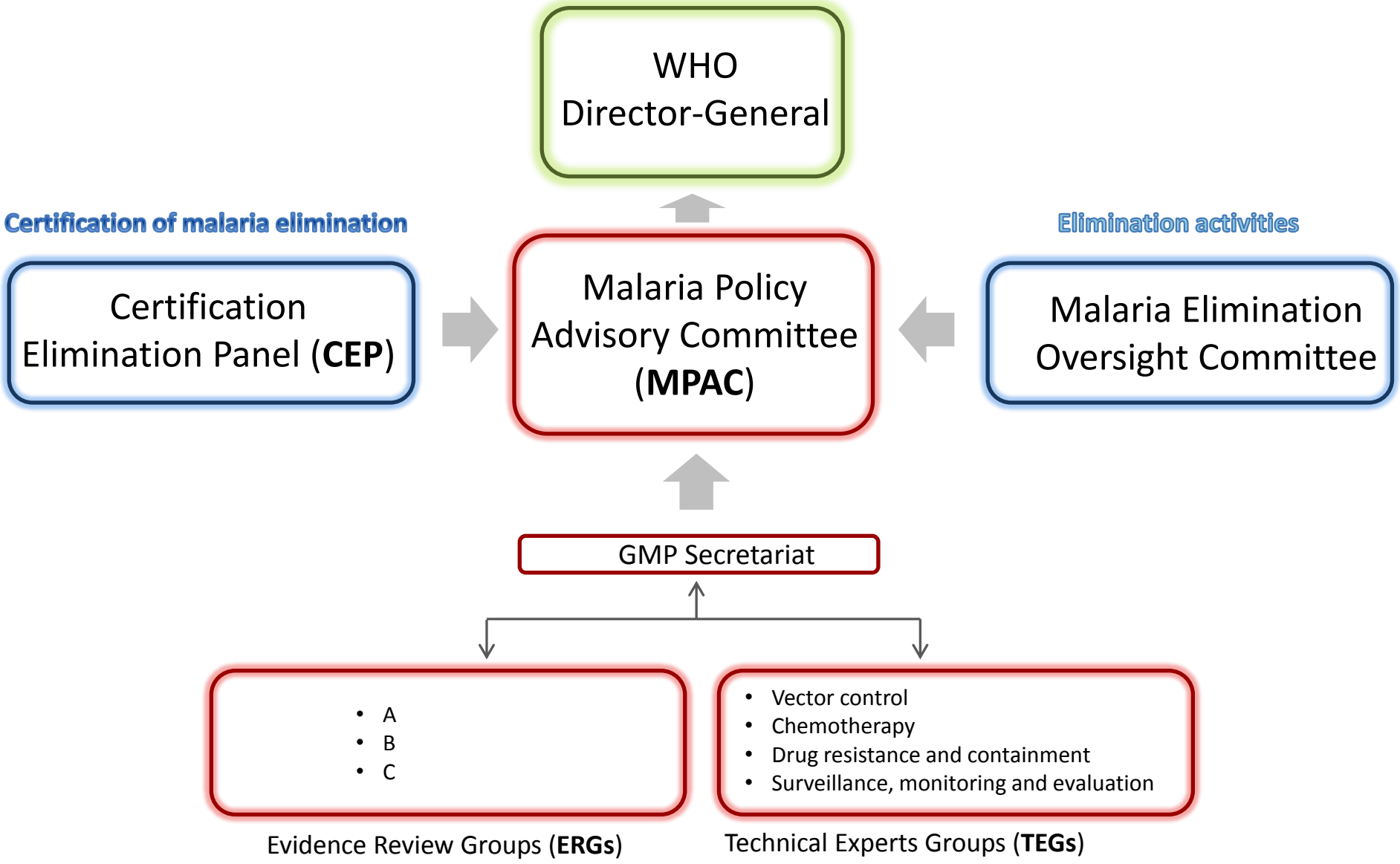
- Norm remains **RDT** or **light** microscopy (without finer/molecular testing techniques).

  - "**RDTs should be available at all levels** in health facilities and community services, while **quality-assured microscopy should be available in hospitals** and **designated** laboratories."
  - "RDTs and microscopy can be used to detect **almost all symptomatic infections** and many, but not all, asymptomatic infections."
  - "More sensitive methods, such as PCR and other molecular techniques …. **are not recommended** for routine case management or surveillance".

WHO certification of (national) malaria elimination

- **Revision from WER, 2014 No. 29:**
  1. Country submits **official request for certification** after **3 past years with zero indigenous** cases
  2. Country formulates **plan of action and timeline** with WHO
  3. Country **finalizes national elimination report** and submits to WHO
  4. Certification Elimination Panel (CEP)
     i. **Reviews national elimination report** and other key documents
     ii. Conducts **field visits to verify** findings
     iii. Develops **final evaluation report**
  5. CEP submits final report to WHO **MPAC with recommendation** to certify now or postpone
     i. CEP may request additional information
  6. WHO MPAC makes **final recommendation to WHO Director-General**
  7. WHO Director-General makes final decision and **officially informs the national government**
     i. Publication in *Weekly Epidemiological Record* and others
     ii. Country listed in WHO *Register of areas where malaria elimination has been achieved*
     iii. Country prevents re-establishment and reports annually to WHO

WHO decision-making process / malaria



CEP – Terms of reference

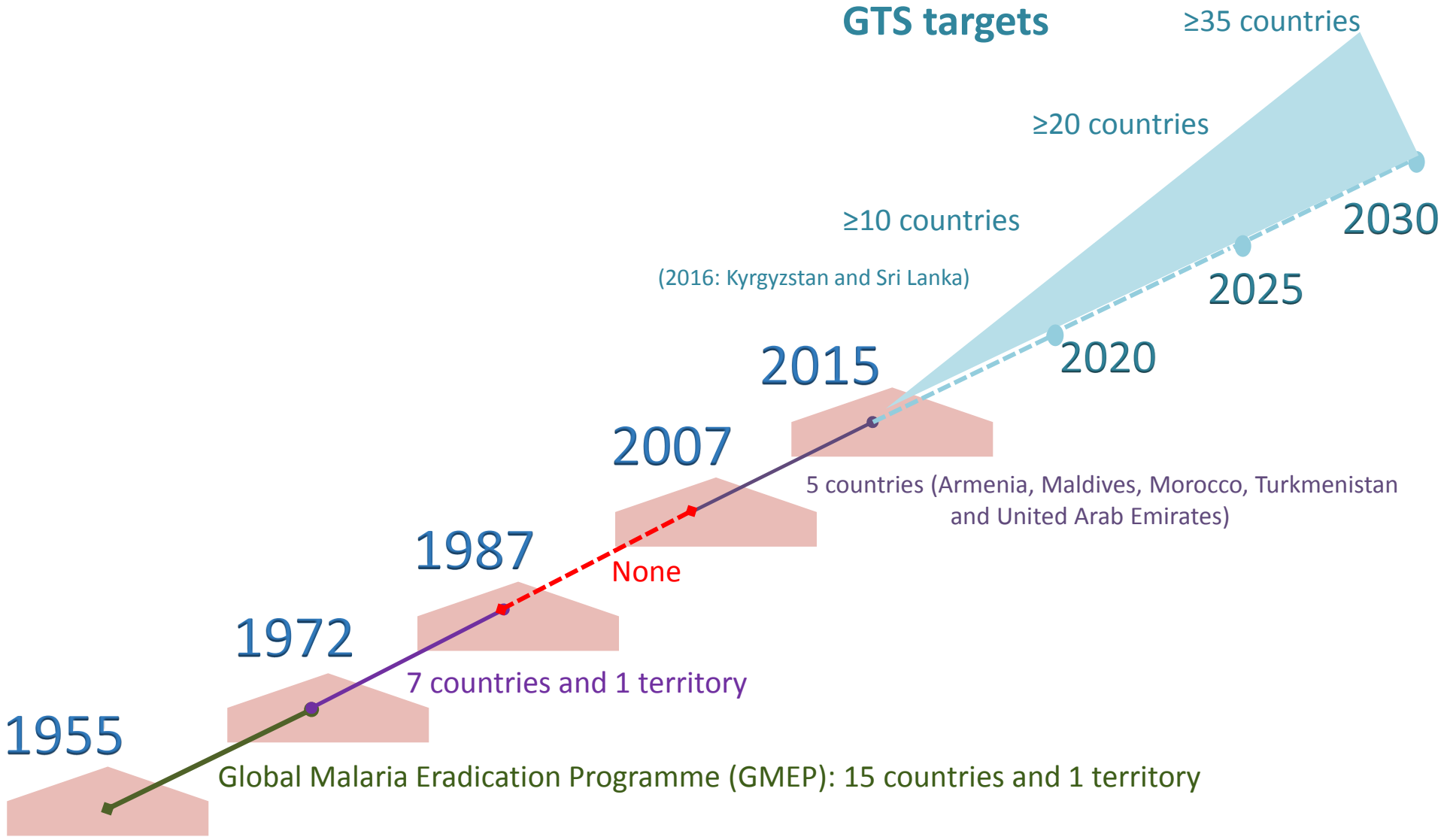
Key roles

- **Review submitted country documentation and national elimination reports**
- **Conduct country assessments and field missions**
  - Review and assess how WHO-proposed procedures and criteria have been applied to document malaria elimination
  - Verify accuracy of the data and information in country documentation and reports
  - Conduct field visits to verify elimination (namely in the last malaria foci)
  - Review national guidelines and plans of action to ensure they are up to date
  - Collect and review any other needed information on the malaria situation in country
  - Assess capacity of the government to maintain its malaria-free status
  - Prepare a final evaluation report for country certification and submit to WHO GMP
- **Review the final evaluation report with all CEP members**
- **Report key findings from the final evaluation report to the WHO MPAC and make a recommendation on certification of malaria elimination**

Composition

- At least eight members, with a WHO-designated chairperson

Overview: countries certified as malaria-free by WHO



Subnational verification of malaria elimination

- Option for large countries that have **interrupted local transmission within certain parts of their territories.**
- Documenting elimination at subnational level should be **as rigorous as the national certification process.**
- Subnational verification should emulate the WHO national-level certification scheme (same criteria and assessment procedures).

Suggested process

- Subnational verification should be subjected to official regulations and/or administrative orders
- Higher-level experienced and independent **national malaria elimination advisory committee** should be established to:
  - i) monitor and verify the work of the programme;
  - ii) help document progress;
  - iii) play a national political and advocacy role for continued efforts.
- Evaluations by independent national teams, incl. international experts, for increased validity and credibility
- Review of documentation and validation of zero incidence of indigenous cases for 3 consecutive years
- Status of subnational verification of malaria elimination to be withdrawn in the event of re-establishment of local transmission

Thank you

Minutes of the Evidence Review Group meeting on the emergence and spread of multidrug-resistant *Plasmodium falciparum* lineages in the Greater Mekong subregion

19–20 December 2016
World Health Organization, Geneva, Switzerland

World Health Organization

Contents

Abbreviations

ACT	artemisinin-based combination therapy
ERG	evidence review group
GMS	Greater Mekong subregion
MDA	mass drug administration
MPAC	Malaria Policy Advisory Committee
Pfcrt	P. falciparum *chloroquine resistance transporter*
Pfdhfr	P. falciparum *dihydrofolate reductase*
Pfdhps	P. falciparum *dihydropteroate synthase*
Pfmdr1	P. falciparum *multidrug resistance protein 1*
PfKelch13	P. falciparum *Kelch propeller domain on chromosome 13*
RSA	ring stage survival assay
SNP	single nucleotide polymorphism

Executive summary

At the request of the Malaria Policy Advisory Committee, an Evidence Review Group conducted a review of the evidence on the spread of multidrug-resistant *P. falciparum* in the Greater Mekong subregion. The regional and global risks this development poses to the efficacy of artemisinin-based combination therapies were assessed in the context of contemporary and historical patterns of emergence and spread of drug-resistant malaria.

Summary conclusions

- Multiple instances of independent emergence and transnational spread of different lineages of artemisinin-resistant parasites have occurred throughout the Greater Mekong subregion (GMS).
- One specific artemisinin-resistant lineage that is dominant at sites in western Cambodia, north-eastern Thailand and southern Lao PDR is also resistant to piperaquine in western Cambodia and north-eastern Thailand.
- These multidrug-resistant parasites have been responsible for increasing dihydroartemisinin-piperaquine failure rates across Cambodia over the last 5 years, rendering this important artemisinin-based combination therapy ineffective in affected areas.
- Artesunate-mefloquine is currently efficacious in Cambodia, with cure rates >95%, and is being used as first-line treatment in Cambodia as an intermediate solution.
- The risk of a highly fit multidrug-resistant lineage spreading widely in higher transmission settings cannot be discounted; however, in the case of artemisinin and piperaquine multidrug resistance, this risk is mitigated by the likelihood that resistance and/or fitness mutations residing on different chromosomes would be rapidly broken up by recombination in multiclonal infections.
- Changing transmission dynamics in Africa have resulted in larger regions of lower malaria transmission intensity similar to the situation in South-East Asia.
- The shift in Africa towards higher drug pressure and less outcrossing increases the potential for the selection and spread of locally generated resistant strains.
- There is also a possibility for parasites from the GMS to potentially become established and spread following importation. Nevertheless, issues of fitness, genetic complexity of the multi-resistant parasites and reduced prevalence of malaria in the GMS mitigate this risk.

Recommendations

- The new data reaffirm the need for an urgent and continued intensive regional malaria elimination campaign in the GMS.
- Surveillance for artemisinin and partner drug resistance needs to be continued and strengthened in the GMS.
- There is a critical need for surveillance outside the GMS to detect potential de novo resistance or the potential introduction of resistant parasites.
- Where surveillance signals a potential threat to leading ACTs, efficacious alternative ACTs should be identified and implemented before resistance reaches critical levels.

1. Rationale

At the Malaria Policy Advisory Committee (MPAC) meeting, 14–16 September 2016, Professor N. White (Mahidol Oxford Tropical Medicine Research Unit) cited new evidence of a multidrug-resistant *P. falciparum* parasite lineage that has developed resistance to both artemisinin and piperaquine in the Greater Mekong subregion (GMS). This lineage has been observed spreading geographically and replacing other *P. falciparum* parasites in the process. WHO called for the new evidence to be submitted for review. MPAC requested that an Evidence Review Group (ERG) assess the relevance of the information and report the potential implications to MPAC at the next meeting in March 2017.

2. Background

Artemisinin resistance in *P. falciparum* has arisen and evolved in the GMS over the past decade. Artemisinin resistance is strongly associated with point mutations in the propeller region of the *PfKelch13* gene. Other genetic changes may also be associated with artemisinin resistance, contributing to resistance and/or compensating for any fitness disadvantage. Initially, many independently arising mutations in *PfKelch13* were observed in the GMS. However, investigation of materials collected over more than a decade shows that the relative frequency of certain mutations has progressively increased to become the dominant mutation in some locations.

Resistance to artemisinin-based combination therapy (ACT) partner drugs, including piperaquine and mefloquine, has emerged in the GMS *(1, 2)*, resulting in the declining efficacy of some of the recommended ACTs. In Cambodia, high treatment failure rates have been observed with dihydroartemisinin-piperaquine, while artesunate-mefloquine currently is highly efficacious. In Viet Nam, dihydroartemisinin-piperaquine has started to show increasing rates of treatment failure. In Thailand, high treatment failures rates have been observed following treatment with artesunate-mefloquine. In areas of southern Lao PDR, the therapeutic efficacy of artemether-lumefantrine has declined.

In response to the drug resistance situation and the declining number of malaria cases, in May 2015, GMS Ministers of Health adopted the *Strategy for malaria elimination in the Greater Mekong subregion 2015–2030 (3)*. The strategy aims to eliminate *P. falciparum* malaria from the GMS by 2025 and all species of human malaria by 2030. The six GMS countries reduced their malaria case incidence by 54% between 2012 and 2015. Reported malaria death fell by 84% over the same period *(4)*. In 2015, a total of 305 027 malaria cases were reported from health facilities and at the community level in the five GMS countries Cambodia, Lao PDR, Myanmar, Thailand and Viet Nam. Of these, approximately 59.7% were *P. falciparum* (~ 182 069 cases).

3. Introduction and declarations of interest

An ERG met 20–21 December 2016 to review new evidence on the emergence and spread of multidrug-resistant *P. falciparum* lineages in the GMS and to advise WHO on the risks posed by artemisinin- and piperaquine-resistant *P. falciparum* parasites.

A list of participants is provided in Annex 1. All ERG members attended the meeting, with the exception of C. Chitnis, K. Marsh and S. Tishkoff. A. Clark participated in the meeting through telephone conference, as he was unable to travel due to bad weather. Organizations invited as observers were the Medicines for Malaria Venture and the Wellcome Trust Centre for Human Genetics, Oxford. The meeting agenda is provided in Annex 2.

All ERG members participating in the meeting submitted a declaration of interest that was assessed by the Drug Efficacy and Response Unit, Global Malaria Programme at WHO. None of the members of the ERG were deemed to have conflicts of interest related to the topics for discussion during the ERG meeting. Unpublished data were presented at the meeting, and discussions were conducted under a confidentiality agreement signed by all participants.

4. Objectives

The primary objective of the meeting was to discuss the emergence and spread of multidrug-resistant *P. falciparum* lineages in the GMS.

Specific objectives

- To review new evidence on the emergence and spread of multidrug-resistant *P. falciparum* lineages with the *PfKelch13* C580Y mutation and the *Pfplasmepsin 2-3* gene amplification in the GMS;
- To assess the risk posed by these parasites in terms of malaria control and elimination in the GMS and in other parts of the world;
- To identify evidence gaps and provide recommendations for further research.

Key definitions

- Artemisinin resistance: partial/relative resistance, described phenotypically as a delay in parasite clearance (in vivo and in vitro).
- ACT resistance: partial resistance to artemisinin plus resistance to a partner drug.
- ACT failure: treatment failure following ACT therapy, regardless of the presence of drug resistance.

5. Process and presentation

The Global Malaria Programme (GMP) convened this ERG meeting to review the evidence and to advice WHO on the risks posed by *P. falciparum* parasites resistant to artemisinin and piperaquine.

Background documents

In preparation for the meeting, WHO collected relevant publications on the topic, and manuscripts were shared by the relevant research groups (Mahidol Oxford Tropical Medicine Research Unit, Bangkok, Thailand, and Southwest Foundation for Biomedical Research, Texas, USA). Annex 3 presents a list of all documents shared by the presenters and provided by WHO.

Presentations

Presentations, followed by a brief discussion, were made by N. White (Mahidol Oxford Tropical Medicine Research Unit, Bangkok, Thailand); T. Anderson (Texas Biomedical Research Institute, San Antonio, USA); A. Dondorp (Mahidol Oxford Tropical Medicine Research Unit, Bangkok, Thailand); and P. Ringwald (World Health Organization, Geneva, Switzerland). Additional information and data were provided by T. Wells (Medicines for Malaria Venture, Geneva, Switzerland); D. Kwiatkowski (Wellcome Trust Centre for Human Genetics, Oxford, UK); and C. Plowe (University of Maryland, Baltimore, USA). Summaries of these presentations are compiled in Annex 4.

6. Evidence reviewed

Situation of drug resistance in the GMS

Multiple instances of independent emergence and transnational spread of different lineages of artemisinin-resistant parasites have occurred throughout the GMS. There are at least three haplotypes of *PfKelch13* C580Y in South-East Asia, although it is not known whether these haplotypes are functionally equivalent. The prevalence of one specific *PfKelch13* C580Y haplotype is increasing, replacing other haplotypes in an area that includes sites in western Cambodia, north-eastern Thailand and southern Lao PDR. This indicates a selective sweep in this part of the GMS.

The fact that different *PfKelch13* C580Y haplotypes are spreading at different rates suggests that additional mutations in these haplotypes confer differences in other fitness attributes. In vitro competition experiments with genetically edited parasites from recently acquired and culture-adapted Cambodian isolates provide evidence that there is no in vitro fitness cost with *PfKelch13* C580Y, unlike *PfKelch13* R539T, which does appear to have a fitness cost (D. Fidock, personal communication). Compared to *PfKelch13* C580Y, the *PfKelch13* R539T mutation mediates a higher level of in vitro resistance, as defined using ring-stage assays (RSAs) with parasites starting at 0–3 hours post-invasion.

Piperaquine resistance is associated with *Pfplasmepsin 2-3* gene amplification. The proportion of parasites resistant to both artemisinin and piperaquine is increasing in Cambodia and areas of Thailand bordering Cambodia. In these areas, it has been found that coincident *PfKlech13* C580Y and increased *Pfplasmepsin 2-3* copy number is associated with dihydroartemisinin-piperaquine treatment failure rates often reaching >25%. In addition to increased *Pfplasmepsin 2-3* copy number, *Pfcrt* could contribute to piperaquine resistance. There may be direct, mechanistic cross-resistance between chloroquine and piperaquine, or compensatory mutations associated with *Pfcrt* that also contribute to the success of piperaquine-resistant strains.

The rapid increase in dihydroartemisinin-piperaquine failure rates across Cambodia over the last 5 years is serious from an operational standpoint. Artesunate-mefloquine currently is efficacious in Cambodia, with cure rates >95%. As an intermediate solution, artesunate-mefloquine is being used as first-line treatment in Cambodia, particularly in areas with high levels of piperaquine resistance.

The use of mass drug administration (MDA) campaigns with dihydroartemisinin-piperaquine in the region could hasten the spread of resistant parasites. In addition, the presence of parasites resistant to dihydroartemisinin-piperaquine limits the drugs available for MDA. Consequently, the use of dihydroartemisinin-piperaquine for MDA should be avoided in situations where dihydroartemisinin-piperaquine is the only available treatment or where high levels of dihydroartemisinin-piperaquine resistance have been reported.

Although malaria is decreasing overall in the GMS, there have been some outbreaks; it is not known whether these outbreaks were in any way caused by resistance. There is a need to continue to strengthen surveillance in the region in order to ensure the quick detection of outbreaks.

Situation of drug resistance outside the GMS

Research has shown that *PfKelch13* mutations can be selected in vitro in an African parasite *(5)*. Accordingly, South-East Asian parasites do not necessarily have a unique feature associated with the emergence of artemisinin resistance. Polymorphisms in *PfKelch13* have been detected at low frequencies globally. There is no evidence of expansion of these parasite lineages, with the possible exception of the independent emergence of *PfKelch13* C580Y mutants in Guyana.

There is evidence of parasites with increased *Pfplasmepsin 2-3* copy number outside the GMS in areas where piperaquine has been used. However, no functional analysis has been performed on these isolates, so there is no proven relationship to piperaquine resistance. So far, there appears to be no relationship between increased *Pfplasmepsin 2-3* copy number and dihydroartemisinin-piperaquine treatment failure outside the GMS.

There are too many unknowns to reliably predict if and when resistance to artemisinin and/or partner drugs (lumefantrine, mefloquine or piperaquine) will become established outside the GMS. However, there is no reason to believe that with sufficient drug pressure, artemisinin and/or partner drug resistance could not become established outside the GMS, either by spontaneous emergence or by importation, and subsequently spread outside the GMS.

Resistance emergence and spread

There are several historical examples of resistance emerging in Asia and spreading to Africa. *Pfdhfr* single and double mutations were able to develop in African parasites. However, the triple mutation never developed spontaneously in Africa, but rather was imported from Asia. Modelling work has shown that

achieving high-level pyrimethamine resistance requires alleles with multiple mutations, the evolutionary trajectories of which are constrained by the need to mediate less drug susceptibility with each new mutation without too great a fitness deficit. Similarly, single mutations in *Pfdhps* evolved and spread in Africa, and just two highly resistant haplotypes containing >2 mutations in *Pfdhps* spread from Asia to Africa *(6)*.

Separate origins of chloroquine resistance in South-East Asia and South America have been established; a single *Pfcrt* chloroquine-resistant allele migrated from South-East Asia to East Africa in the 1970s and became established across the continent.

It is difficult to reconstruct from historical data the exact sequence of events that caused Asian antimalarial drug-resistant parasites to spread to Africa. Although the evolution of artemisinin resistance can be observed in real time in South-East Asia, it may not be possible to completely understand the potential for spread outside the GMS before it is too late. Thus, an urgent and continued intensive regional malaria elimination campaign in the GMS is needed.

It should be noted that since the spread of chloroquine resistance and sulfadoxine-pyrimethamine resistance to Africa, many features of the malaria epidemiology and health systems in Africa and Asia have changed.

It may be that conditions in Asia are particularly suited to the development of successful resistant parasites, which are subsequently able to invade Africa. There may be epidemiological barriers to the development of antimalarial resistance in Africa, such as multiple infection and increased rates of outbreeding. Nevertheless, historical examples indicate that if a resistant parasite is imported from elsewhere, it can spread. Therefore, the risk of a highly fit multidrug-resistant lineage spreading widely in higher transmission settings cannot be discounted. However, such a risk is mitigated by the likelihood that multilocus extended haplotypes, especially those involving resistance and/or fitness mutations on different chromosomes, would be rapidly broken up by recombination in multiclonal infections. Thus, the risk of transnational spreading of parasites with both artemisinin resistance (encoded by mutations on chromosome 13) and resistance to piperaquine or mefloquine (likely encoded to an important extent by copy number variation on chromosomes 14 and 5, respectively) may be lower in moderate- and high-transmission areas of South-East Asia and sub-Saharan Africa than in the GMS. This is especially the case when the drug pressure in these areas of high transmission is relatively low.

Notably, it took several years from the emergence of *PfKelch13* mutations to the point at which they became clinically relevant and detected at significant prevalence in field surveys. This suggests that there was a latent period during which additional compensatory adaptations were acquired, enabling the parasites to spread; the *PfKelch13* C580Y selective sweep may be the most recent evidence of this process. For *PfKelch13* mutations, fitness costs seem to be greater in older isolates than in more recent isolates, also indicating the accumulation of additional compensatory mutations (D. Fidock, unpublished data). The later introduction of ACTs in Africa relative to Asia may explain the lack of *PfKelch13* mutations expanding in the population.

The successful establishment of a resistant parasite reduces variation around the genetic target. This loss of variation could be a fitness disadvantage if the environment were to change, for example by switching antimalarial therapy or changing transmission dynamics. In the absence of drug pressure, for instance, both increased *Pfmdr1* copy number in Cambodia and chloroquine-resistant *Pfcrt* in Africa appear to be lost.

Although the artemisinin resistance phenotype is limited to one single life cycle stage at present (the early ring stage), there is the potential that, in the future, artemisinin resistance in other *P. falciparum* life cycle stages in humans will emerge.

For *P. vivax*, no artemisinin-resistant strains and no *PvKelch13* mutations have been reported. Artemisinins have greater activity against *P. vivax* than against *P. falciparum*, and *P. vivax* has a much lower parasite biomass than *P. falciparum*. Therefore, compared to *P. falciparum*, the capacity for de novo resistance selection in *P. vivax* is relatively low.

7. Conclusions and recommendations

The ERG addressed the following key questions and made the following conclusions and recommendations for consideration:

Is there new evidence of selection and spread of specific artemisinin-resistant genotype(s) in the GMS?

There is evidence that selective sweeps at *PfKelch13* have occurred throughout the GMS. Currently, the *PfKelch13* C580Y mutation can be found in several genetic backgrounds (haplotypes) throughout the GMS. The prevalence of one specific *PfKelch13* C580Y haplotype is increasing, replacing other haplotypes in an area that includes sites in western Cambodia, north-eastern Thailand and southern Lao PDR. This indicates a selective sweep in this part of the GMS. However, the frequencies of different *PfKelch13* C580Y haplotypes vary by region, and no single haplotype is dominant throughout the GMS. The emergence and spread of *PfKelch13* C580Y haplotypes is a dynamic process, and continued surveillance is essential in order to detect the emergence of a region-wide sweep of a single haplotype.

Although some *PfKelch13* mutants, in absence of partner drug resistance, have increased gametocytaemia compared to wild-type parasites, it is not clear whether this characteristic actually increases transmission. Membrane feeding studies would be able to determine whether there is a significant transmission advantage with the various *PfKelch13* mutations.

If yes, what would be the consequences of the selection and spread of specific artemisinin-resistant genotype(s)?

The consequences of the selection and spread of specific artemisinin-resistant genotype(s) could include:

- Partial or total loss of efficacy to artemisinin treatments;
- Global spread of a dominant haplotype that would increase levels of resistance (following the history of chloroquine resistance and sulfadoxine-pyrimethamine resistance);
- A common genetic background could accumulate mutations at other (i.e., loosely or unlinked) loci that might encode potential compensatory factors, such as ACT partner drug resistance, fitness or transmissibility;
- Alternatively, the spread of *PfKelch13* C580Y haplotypes could result in a loss of within-population genetic diversity. This loss of variation could become a fitness disadvantage for *P. falciparum* should the environment change, for example, by switching antimalarial therapy or changing transmission dynamics.

Is there evidence that artemisinin resistance has facilitated the emergence of partner drug resistance in the GMS? If yes, for which partner drug(s)?

There is evidence of parasites with resistance to both artemisinin and piperaquine in Cambodia and areas of Thailand bordering Cambodia. Piperaquine resistance has occurred in the past in China and Cambodia independently of artemisinin resistance. Therefore, artemisinin resistance is not a prerequisite for the initial appearance of piperaquine resistance.

In the GMS, the perception is that piperaquine resistance occurred after the initial discovery of artemisinin resistance; however, it is not possible to determine the temporal relationship between the emergence of either resistance (i.e., piperaquine resistance could have already been present at low levels). The molecular and physiological mechanisms of piperaquine and artemisinin resistance appear to be independent. There is no evidence that *PfKelch13* C580Y confers any resistance to piperaquine. Instead, the mechanism of piperaquine resistance appears to involve gene amplification of *Pfplasmepsin 2-3*. This type of genetic change (i.e., copy number variation) is unstable and allows rapid back mutation to single copy, particularly during meiosis. As a result, the temporal tracking of piperaquine resistance is difficult.

Is there evidence that artemisinin resistance has facilitated the selection and spread of partner drug resistance in the GMS? If yes, for which partner drug(s)?

Recent results clearly show the spread of dual-resistant phenotypes and genotypes (i.e., resistant to both piperaquine and artemisinin) in Cambodia, where dihydroartemisinin-piperaquine was the first-line treatment for *P. falciparum* malaria. As the resistance mechanism is not understood, it is difficult to understand the spread of resistance in the different genetic backgrounds. It is possible that there is an epistatic effect[a] that changes the synergisms between the two types of drug resistance in different genetic backgrounds, but there is as yet no evidence of this hypothesis.

Different factors affect the selection and spread of piperaquine resistance:

- Artemisinin resistance may increase the exposure of the parasite population to piperaquine;
- Since piperaquine has a long half-life, after treatment with dihydroartemisinin-piperaquine, piperaquine is effectively present as a monotherapy for about 1 month. During this time, exposure to piperaquine may be low enough to allow the survival of piperaquine-resistant merozoites emerging from the liver, further fostering the selection of resistance;

Conversely, it is possible that the reduced efficacy of piperaquine has facilitated the selection of artemisinin-resistant mutations.

With regard to the selection and spread of resistance to partner drugs other than piperaquine:

- Artesunate-pyronaridine: Not enough data are available to draw any conclusions;
- Artemether-lumefantrine: Efficacy was always suboptimal in Cambodia and in Thailand; consequently, few data are available for these countries. Artemether-lumefantrine is still highly efficacious in Myanmar, and was so in Lao PDR until recently;
- Artesunate-mefloquine: As there was a background of mefloquine resistance in the GMS prior to the introduction of artemisinin drugs, it is not possible to assess the role of artemisinin resistance in the emergence and selection of mefloquine resistance. Recent data demonstrate that the use of dihydroartemisinin-piperaquine has coincided with a reversion of mefloquine resistance to sensitivity. It is not known whether this is because mefloquine is no longer used as a treatment, or whether this is a direct consequence of dihydroartemisinin-piperaquine treatment. The recent decision to use artesunate-mefloquine as first-line treatment in Cambodia will enable us to determine whether this treatment results in an increase in mefloquine resistance in the parasite population, particularly in populations where mefloquine resistance was previously prevalent. This could provide key information for the utility of alternating drug strategies in combating resistance.

Risk factors for the development of ACT partner drug resistance include use of partner drugs as monotherapy; a long half-life of the partner drug; resistance to artemisinin; and non-adherence or use of substandard drugs, particularly for combination therapy, resulting in inadequate dosage of the partner drug to entirely clear the parasite load. Little analysis of these risk factors has been done.

Is there evidence of the geographical extent of artemisinin resistance outside the GMS?

No evidence was presented to indicate artemisinin resistance outside the GMS. There is evidence of multiple *PfKelch13* mutations in many geographic regions. However, none of these are associated with a haplotypic expansion of *PfKelch13*. One exception is the possible independent emergence of *PfKelch13* C580Y in Guyana.

[a] Epistasis is where the phenotypic effect of one mutation differs depending on the presence of another mutation.

What is the risk (and risk factors) of the spontaneous emergence, selection and spread of artemisinin resistance and/or resistance to ACT partner drugs outside the GMS?

Historically, chloroquine, sulfadoxine and pyrimethamine resistance emerged in South-East Asia, and those drug-resistant haplotypes spread to Africa. Thus, there is an historical precedent to support concerns that this could happen for artemisinin resistance and/or for resistance to a partner drug(s).

Nevertheless, mefloquine resistance also emerged in the GMS, but it has not spread to other regions, most likely because mefloquine has not been used extensively outside of the GMS. In addition, low-level resistant haplotypes encoded by single mutations in *Pfdhfr* and *Pfdhps* have had multiple African origins and were not acquired from South-East Asia.

There may be epidemiological barriers to the development of antimalarial resistance in Africa, such as multiple infection and increased rates of outbreeding. Although the risk of a highly fit multidrug-resistant lineage spreading widely in higher transmission settings cannot be discounted, this risk is mitigated in the case of artemisinin and piperaquine multidrug resistance by the likelihood that resistance and/or fitness mutations residing on different chromosomes would be rapidly broken up by recombination in multiclonal infections.

It may be that conditions in Asia are particularly suited to the development of successful resistant parasites, which are subsequently able to invade Africa. However, changing transmission dynamics in Africa have resulted in larger regions of lower malaria transmission intensity similar to the situation in South-East Asia. The shift in Africa towards higher drug pressure and less outcrossing increases the potential for the selection and spread of locally generated resistant strains. There is also a possibility for parasites from GMS to potentially become established and spread following importation. Nevertheless, issues of fitness, genetic complexity of the multi-resistant parasites and reduced prevalence of malaria in the GMS mitigate this risk.

The selection and spread of drug resistance in the GMS is presumably related to the high drug pressure that has been present in the region over an extended period of time (particularly for dihydroartemisinin-piperaquine in Cambodia), and to the historical use of monotherapy in South-East Asia, which selected for specific drug-resistant variants.

PfKelch13 mutations are present at low frequencies in *P. falciparum* outside the GMS. This includes the *PfKelch13* C580Y mutation that has been found in Africa and elsewhere. However, the fact that the *PfKelch13* C580Y mutation exists outside the GMS but is not spreading suggests that additional mutations may be necessary to modulate the potential fitness costs of the *PfKelch13* C580Y mutation or may reflect the relatively lower exposure of parasite populations to artemisinin derivatives. Under drug pressure, however, the necessary compensatory mutations might be acquired, enabling the spread of artemisinin-resistant parasites of African origin.

Unlike the complex loci (multiple mutations) needed for clinically relevant levels of chloroquine and sulfadoxine-pyrimethamine resistance, data from the GMS indicate that the primary resistance mutations for artemisinin, piperaquine and mefloquine are single nucleotide polymorphisms (SNPs) or copy number variations. Consequently, there is a high probability that copy number variations of *Pfplasmepsin 2-3* and *Pfmdr1* needed for clinically relevant resistance key ACT partner drugs already exist at low frequencies in the parasite population outside the GMS. These parasite populations could remain at low frequencies or disappear in the absence of selecting factors. Nevertheless, there is the potential that these variants may rapidly select and spread under increased drug pressure. The massive and uncontrolled use of dihydroartemisinin-piperaquine in settings outside the GMS may thus lead to resistance and loss of efficacy of this treatment, even if resistant parasites are not imported from the GMS.

Overall, there is a significant risk of artemisinin and partner drug resistance outside the GMS – either via spontaneous emergence or importation, and spread. Therefore, resistance surveillance in regions outside the GMS is critical.

To summarize, risk factors contributing to the potential for resistant strains to emerge locally or for imported resistant parasites to spread are multifactorial and interdependent (Table 1).

Table 1. Factors affecting the potential for resistant strains to emerge or for imported resistant parasites to spread to Africa

	Increase the potential	Decrease the potential
Health system	Poor access to diagnostics and quality drugs	Improvement in access to diagnostics and quality drugs
Antimalarial drugs	Widespread use of antimalarial drugs, for example MDA; use of substandard antimalarial drugs, available primarily on the private markets; treatment non-adherence	Limited use of antimalarial drugs in immune adult populations and asymptomatic carriers
Human	Increased population movement between Africa and Asia; increased exposure to mosquitoes; low immunity	Reduction in malaria in the GMS[b] potentially leading to a reduction in migration of infected people; reduced exposure to mosquitoes;
Parasite population structure	Changing (decreasing) malaria prevalence leading to changes in parasite population structure and the consequent reduction of the possibility of multiple infection, limiting the outbreeding between different genotypes	Extensive parasite diversity in African parasite populations and evidence of outcrossing in parasite populations, even in regions of low transmission
Vector	The ability of African vectors to transmit Asian parasites; receptivity in areas where importation happens	Greater vector control measures lowering receptivity
Parasite	High transmission potential of *P. falciparum*-resistant strains; low fitness costs of imported versus local strains	Low transmission potential of *P. falciparum*-resistant strains; high fitness costs of imported versus local strains

Identify research questions that might improve our understanding of artemisinin resistance, the selection of specific artemisinin-resistant genotypes, and the role that artemisinin resistance plays in the emergence and selection of resistance to ACT partner drugs.

1. Biologically characterize artemisinin resistance mutations and mechanisms of artemisinin resistance – both as they occur and using CRISPR/Cas9 to study mutations in a single genetic background:

- Measure fitness both in vitro and using longitudinal population data in vivo;
- A standing hypothesis is that artemisinin resistance is primarily conferred by *PfKelch13* mutations, and other aspects of fitness are modulated by the genetic background. However, this hypothesis needs to be tested and quantified by performing whole-genome sequencing of defined strains in order to determine relevant mutations for testing: (i) by examining the dose-response curve of

[b] In recent years, the access to malaria treatment and reporting of malaria have improved significantly. In 2015, a total of 305 027 malaria cases were reported from health facilities and at the community level in the five GMS countries Cambodia, Lao PDR, Myanmar, Thailand and Viet Nam. Of these, approximately 59.7% were *P. falciparum* (~ 182 069 cases). In 2015, the estimated total number of cases in the five countries was 515 015 (range: 360 000–764 000) (World Malaria Report 2016). In 2010, the five countries reported 858 713 malaria cases, of which approximately 69.9% were *P. falciparum* (~ 600 458 cases). In 2000, the five countries reported 1 418 098 malaria cases, of which approximately 83.5% were *P. falciparum* (~ 1 184 253 cases). In 1990, the five countries reported 1 532 558 malaria cases, of which approximately 81.1% were *P. falciparum* (~ 1 243 103 cases) (World Malaria Report 2011). Data reporting before 1990 is limited.

each parasite life stage with respect to the drug; (ii) by determining the genetic components of fitness in competitive growth studies;

- Measure the effect on transmissibility of the specific mutation and the genetic background in both Asian and African vectors;
- Explore mechanisms of action for a better understanding of how resistance emerges and spreads. A hallmark of this resistance, in contrast to other known resistant mechanisms, is that multiple mutations (>25 SNPs associated with delayed clearance but not necessarily validated as markers of artemisinin resistance) in the *PfKelch13* propeller domain result in a resistance phenotype;
- Explore whether this is a loss-of-function mechanism and identify the underlying pathways;
- From the whole-genome sequencing analysis, develop a targeted set of genetic markers across the genome: (i) include both fitness and molecular markers; (ii) focus on new, emerging resistance markers;
- Validate a better assay as a surrogate of phenotypic resistance. Both RSA and clearance time estimation are limited to detecting ring stage activity, and neither are useful in large-scale surveillance (i.e., RSAs require specialist laboratories, and clearance times require frequent observations of single patients);
- There is some evidence of delayed clearance that is not obviously *PfKelch13*-mediated. There is a need to understand or find other markers that produce this phenotype.

2. Investigate the impact of parasite population characteristics, such as population genetic structure, on the emergence and spread of drug resistance using modelling approaches and resistance mechanism interactions; the simplicity of the *PfKelch13* mutations that result in resistance raises the question as to why resistance has emerged and spread in the GMS and not elsewhere.

3. Assess the role of human mobility and drug use in the emergence and spread of resistance:

- Explore alternative epidemiological approaches to surveillance, for example, using social scientific approaches to identify the movement of human populations at risk of transporting resistant parasites from Asia to Africa;
- Study special populations with little access to health facilities.

4. Explore alternative drug regimens: As new drugs are unlikely to be available within the next 5 years, existing drugs should be evaluated for use in MDA and as treatment. This could include a re-examination of atovaquone-proguanil; in particular, investigate whether *cytochrome b* atovaquone resistance mutations are transmissible.

5. Determine the contribution of artemisinin resistance to the spread of multidrug-resistant malaria parasites:

- Explore the potential impact of the increased use of ACTs in Africa on the emergence and spread of artemisinin resistance.

6. Investigate the contribution of partner drug resistance to the spread of multidrug-resistant parasites:

- Determine whether the loss of *Pfmdr1* copy number following the replacement of artesunate-mefloquine with dihydroartemisinin-piperaquine in Cambodia has been because of competing drug resistance mechanisms and/or the removal of mefloquine drug pressure;
- Explore the potential impact of the increased use of dihydroartemisinin-piperaquine in Africa, particularly in MDA, on the emergence and spread of artemisinin and piperaquine resistance.

7. Conduct thorough and well-coordinated surveillance to enable early identification of the spread of resistant strains and to enact changes in drug strategies that may delay the spread. Faster identification of resistant mutations, haplotypes or strain(s) allows for an earlier start to characterizing the mechanism of resistance. Priorities for surveillance for genetic evidence of artemisinin resistance inside and outside the GMS include:

- Active engagement of Ministries of Health/national malaria control programmes;
- Active surveillance, especially in areas of decreasing transmission or under MDA;
- Coordinated sampling and sharing of data in real time from research groups and nongovernmental organizations to Ministries of Health/national malaria control programmes and WHO;
- Continued and extended surveillance for the emergence and spread of partner drug (lumefantrine, mefloquine or piperaquine) resistance in Africa; this is particularly important in countries where dihydroartemisinin-piperaquine is used for treatment (in private or public sector) or being used as MDA;
- Identification of potential evidence of Asian parasitic genetic backgrounds in the African setting; the gold standard would be whole genome sequencing of *Plasmodium* from multiple regions.

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Annex 2: Agenda

Monday 19 October 2016		
09:00–09:15	Welcome	P. Alonso, Director GMP D. Wirth, Chair ERG
09:15–09:20	Objectives of the meeting and declaration of interest	P. Ringwald
09:20–10:00	Intrahost selection and spread of antimalarial drug resistance (basic principles-PK-PD-ACT rationale)	N. White
10:00–10:45	Evolution of antimalarial drug resistance (population genetics principles, selective sweeps, history of chloroquine and SP resistance emergence and spread, longitudinal studies on the Thai–Myanmar border)	T. Anderson
10.45–11.15	*Coffee/tea break*	
11:15–12:00	Development of artemisinin and partner drug resistant falciparum malaria in the GMS (emergence and spread of artemisinin and partner drug resistance in the GMS, recent transnational spread)	A. Dondorp
12:00–12:45	World-wide situation of drug efficacy and drug resistance outside GMS	P. Ringwald
12:45–14:00	*Lunch*	
14:00–14:30	Questions and answers from review panel	
14:30–16:00	Discussion	
16:00–16:30	*Coffee/tea break*	
16:30–18:30	Discussion (continued)	
Tuesday 20 December 2016		
09:00–12:30	Formulation of ERG recommendations (Closed session)	D. Wirth, Chair ERG
12:30	Closing remarks (Closed session)	P. Alonso

Annex 3: Supporting documents

Documents shared by presenters

Anderson TJ, Nair S, McDew-White M, Cheeseman IH, Nkhoma S, Bilgic F, et al. Population parameters underlying an ongoing soft sweep in Southeast Asian malaria parasites. Mol Biol Evol. 2017;34(1):131–44 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5216669/).

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Annex 4: Presentations

Intrahost selection and spread of antimalarial drug resistance (N. White)

Antimalarial drugs reduce parasite multiplication, with activity against specific stages of the malaria parasite life cycle. Antimalarial pharmacokinetics and pharmacodynamics are diverse**.** Clearance half-lives vary from less than 1 hour (artemisinins) to around 1 month (chloroquine). Different parasite clearance time profiles reflect differing pharmacodynamics; of clinically available agents, artesunate has the most rapid parasite clearance time. Drug resistance causes the dose-response curve to shift to the right and may also change the shape of the curve. As the parasite drug resistance level increases, recrudescence occurs earlier following treatment, and drugs can eventually lose all activity.

Antimalarial drugs differ in their propensity to generate de novo resistance. For example, although highly potent against susceptible parasites, proguanil resistance is rapidly generated and selected, is transmissible, and the consequent malaria infection is refractory to treatment. Similarly, resistance to pyrimethamine, mefloquine or atovaquone is readily generated and selected. However, the barriers to resistance for some antimalarial drugs (quinine, artemisinin) appear to be higher, requiring more extensive and sustained drug pressure.

Most identified mechanisms of antimalarial drug resistance involve genetic mutation. Resistance mechanisms include changes to the target (*Pfdhfr, Pfdhps, Pfplasmepsin 2-3, cytochrome b, PfATPase4*), mutation or amplification of drug pumps (*Pfcrt, Pfmdr1*), and unknown mechanisms (*PfKelch13, Pfcarl*).

Transmission of de novo resistance requires recrudescence. Therefore, treatment failures drive the spread of resistance. There is some evidence of increased gametocytaemia following the initial treatment of resistant infections, which may facilitate the spread of resistant strains.

De novo resistance is most likely to arise and subsequently spread from a hyperparasitaemic individual receiving inadequate treatment. Such patients have more parasites and consequently more genetic variation in their parasite population; moreover, they have inadequate host defense against the infection. In addition, treatment failure is more likely in hyperparasitaemic individuals with the potential for transmission of resistant parasites.

Symptomatic malaria is generally caused by between 10^7 and 10^{12} parasites. The rationale for ACTs is that the artemisinin reduces parasite numbers quickly to around 10^4, while the partner drug kills the remaining parasites and provides protection over several parasite cycles. For ACTs, combination therapy protects the artemisinin and partially protects the partner drug against de novo selection of resistance owing to the reduction in parasitaemia achieved with the artemisinin. However, once artemisinin resistance develops, the unprotected partner drug is exposed to larger numbers of parasites, accelerating the selection of partner drug resistance. The long terminal elimination phase of partner antimalarial drug drugs provides a selective filter that amplifies the spread of resistance to the partner compound.

Evolution of antimalarial drug resistance (T. Anderson)

The selection of antimalarial drug resistance can be considered:

- 'Hard', whereby genetic variation around the selected gene is purged, thereby producing a strong signature in the genome that is easy to find by association; or
- 'Soft', whereby multiple genetic backgrounds are associated with the selected gene, leaving a weak signature in the genome that is difficult to find by association.

Hard selection events are more easily detected and tracked than soft events. As a result, the initial emergence of resistance as a soft event may go undetected until more competitive variants begin to replace less competitive ones. A hard event becomes evident as diverse genetic backgrounds are purged. Whether an event appears hard or soft, therefore, may depend on when and where you look.

Pyrimethamine: Low-level resistance to pyrimethamine is easily generated. Thus, multiple independent emergences of pyrimethamine resistance would be expected. However, data from South-East Asia show that, for parasites with two or more *Pfdhfr* mutations, variation around *Pfdhfr* is very low, with a single dominant microsatellite haplotype associated with resistant *Pfdhfr* alleles across five South-East Asian countries *(7)*. This indicates a hard selective sweep at this locus and a single origin of resistant alleles. These strains that are highly resistant to pyrimethamine subsequently spread from South-East Asia to Africa *(8)*, mirroring the emergence and spread of chloroquine resistance *(9)*.

It is not clear why high-level resistance has so few origins. In every malaria patient, an estimated 100–1000 parasites contain point mutations at each position in the genome. However, although resistance mutations may be common, successful resistance alleles are rare. Perhaps this is because multiple simultaneous changes are required for resistance to arise or because compensatory mutations are needed elsewhere in the genome.

Artemisinin: The emergence and spread of artemisinin resistance in the Thai–Myanmar border area allows for the examination of an ongoing selective event. In 2014, around 90% of parasites had *PfKelch13* mutations. The sequencing of *PfKelch13* (*n* = 1876), genotyping of 75 flanking SNPs and investigation of parasite clearance rates (*n* = 3552) revealed 32 independent coding mutations, including those outside the propeller region, associated with significant reductions in the parasite clearance rate *(10)*. These represent soft selective events.

The first *PfKelch13* mutations along the Thai–Myanmar border were described in 2003. Allele diversity increased until 2012, with the *PfKelch13* E252Q mutation dominating until 2010. However, since the emergence of *PfKelch13* C580Y in 2006, it has progressively replaced other mutations to become the dominant mutation, present in around 70% of parasites in 2014 *(10)*. Thus, the selection signature is becoming harder as *PfKelch13* C580Y apparently outcompetes other variants. Whether this will lead to the spread of the *PfKelch13* C580Y allele outside of the GMS is unknown, but the experience of pyrimethamine and chloroquine resistance suggests that this is a possible scenario.

Many different *PfKelch13* mutations result in phenotypic artemisinin resistance. As other mutations are associated with greater delays in parasite clearance rates, the relative selective advantage of *PfKelch13* C580Y is unclear. Furthermore, around 4% of *PfKelch13* wild-type alleles have extended parasite clearance times, indicating the possibility that other loci or contributing factors may be involved in artemisinin resistance *(10)*.

The large mutational target size for *PfKelch13* (87–163 base pairs) makes multiple origins of resistance likely. In addition, the frequency distribution of artemisinin-resistant alleles leads to an estimated short-term effective parasite population size of 88 000 to 1.2 million. This figure is greater than previously estimated and indicates a higher adaptive capacity in *P. falciparum*. In light of this, to avoid the development of antimalarial drug resistance, combination therapies need to be more complex.

PfKelch13 mutations are currently being detected in Africa and elsewhere, but have not yet become established or begun to spread. The reasons for this are unclear, but may depend on the fitness costs of resistance mutations, as well as higher transmission, lower drug pressure, differences in the response of the local vectors, the higher proportion of multiple genotype infections leading to stronger intrahost competition, and the predominance of outbreeding and high recombination rates, resulting in the breaking up of multilocus genotypes.

Other loci show a temporal change in frequency similar to that of *PfKelch13* and may also be associated with artemisinin resistance. The strongest signature was an SNP in phosphatidylinositol 4-kinase (PI4K) *(11)*. However, no functional validation of this locus has been performed, for example using CRISPR/Cas9.

Conclusion

There are clear historical examples of antimalarial drug-resistant parasites arising in Asia and spreading to Africa, in some cases replacing indigenous alleles. As we do not fully understand the process by which this happens, the intercontinental spread of artemisinin-resistant parasites also appears to be a possibility. Therefore, the elimination of South-East Asian parasite populations should be prioritized, as this would remove key sources of artemisinin and piperaquine resistance.

Discussion

The studies discussed were representative of the population along the Thai–Myanmar border. The relative frequencies of mutations appear to be similar in hyperparasitaemic patients, those with 'normal' parasite loads, and asymptomatic individuals identified using quantitative real-time polymerase chain reaction (qPCR). The majority of malaria parasites in the region carry *PfKelch13* mutations. Owing to malaria elimination efforts, there were very few cases of *P. falciparum* malaria in this specific region (Thai–Myanmar border) in 2015–2016, reducing the potential for spread to other regions. However, the potential remains for other resistant haplotypes to develop elsewhere independently.

The spread of certain *PfKelch13* mutants is dependent on whether they affect the recrudescence rate; there is some evidence that gametocytaemia may also be increased with these mutations.

Development of artemisinin- and partner drug-resistant falciparum malaria in the GMS (A. Dondorp)

When ACTs were introduced as first-line therapy along the Thai–Myanmar border, it was against a background of widespread antimalarial drug resistance to chloroquine, sulfadoxine-pyrimethamine, mefloquine and (to a lesser extent) quinine ± tetracycline. Artesunate-mefloquine was introduced in the early 1990s at a time when mefloquine resistance was at quite a high level, but despite this the combination remained efficacious for >15 years. Drug pressure on the artemisinins and partner drugs in the GMS is in part derived from the poor quality of drugs and the availability of artemisinin monotherapy. Artemisinin resistance, evident phenotypically as a delay in parasite clearance, was described in 2009 in western Cambodia; however, it was retrospectively identified as having already been present at the beginning of the millennium, at a time when higher than expected ACT failure rates were intermittently reported in the same areas. Declining clinical efficacy with artesunate-mefloquine resulted in a change to dihydroartemisinin-piperaquine in western Cambodia in 2008 and elsewhere in 2010, although this transition was not complete until 2012.

Artesunate-mefloquine: In Cambodia, infections from parasites with *PfKelch13* C580Y and a single *Pfmdr1* copy number had a 100% cure rate. Along the Thai–Myanmar border, artesunate-mefloquine cure rates were the lowest (~60%) in patients with multiple *Pfmdr1* copies and any *PfKelch13* propeller region SNP. In addition, cure rates declined as the proportion of *PfKelch13* mutations increased (observed in patients with single *Pfmdr1* copy number, although this does not exclude low-level mefloquine resistance caused by other mechanisms). By 2013, around 84% of infections had *PfKelch13* mutations and 65% had multiple *Pfmdr1* copies *(12)*.

Dihydroartemisinin-piperaquine: Piperaquine has a long half-life, and because of its bi-phasic pharmacokinetic profile, a small increase in the minimum inhibitory concentration can lead to a large reduction in the time the parasite is exposed to parasitocidal drug concentrations. Resistance to dihydroartemisinin-piperaquine has rapidly emerged in western Cambodia, with cure rates of <70% in Pursat in 2012–2013 *(13)*. Resistance appears to be spreading from western Cambodia to eastern Cambodia, and increases in the proportion of parasites with *PfKelch13* mutations appear to have preceded the emergence of piperaquine resistance *(14)*. However, of note, there was some use of piperaquine in western Cambodia around 2000 to 2003, and piperaquine resistance was likely to have pre-existed at low levels in the region.

Increased *Pfplasmepsin 2-3* copy number predicts dihydroartemisinin-piperaquine treatment failure and has been closely associated with in vitro piperaquine resistance. In 2014–2015, the proportion of parasites with both *PfKelch13* and *Pfplasmepsin 2-3* copy number amplification had increased to around 50% in Cambodia. Treatment failure rates with these parasites are around 35% at day 28 and 65% at day 42. The *Pfplasmepsin 2-3* amplification is almost invariably observed in association with *PfKelch13* mutated parasites.

ACT efficacy: Even in the presence of artemisinin resistance, efficacy rates may still be adequate. It is only once resistance to the partner drug emerges that treatment failure rates increase significantly. Artemisinin resistance, as indicated by an increase in day-3 slide positivity rates, is expanding across the GMS *(14)*. Treatment failure rates are >10% to four ACTs in Cambodia; two ACTs in Thailand, Lao PDR and Viet Nam; and one ACT in Myanmar, India and China (Yunnan Province). Increased ACT failure rates are driving the onward transmission of resistant parasites, facilitating the rapid spread of resistance. In addition, gametocytaemia increases with artemisinin resistance, as well as with mefloquine resistance and possibly piperaquine resistance, which potentially increases transmission *(15)*.

There is some suggestion that piperaquine and mefloquine may have opposing resistance mechanisms, with the potential for drug cycling to combat resistance. Furthermore, these opposing effects have been attributed to changes in *Pfmdr1* copy number: Whereas mefloquine resistance seems to be associated with an increased *Pfmdr1* copy number, piperaquine resistance seems to be found when the *Pfmdr1* copy number is 1. However, it cannot be excluded that the disappearance of mefloquine resistance in areas where dihydroartemisinin-piperaquine is used may be caused by the removal of drug pressure, as gene amplifications are easily lost. A trial of triple therapy with dihydroartemisinin-piperaquine-mefloquine is being conducted, which may inform this discussion.

Artemisinin resistance: The area of South-East Asia where *PfKelch13* mutations have been detected is expanding *(16)*. There is variation in the dominant phenotype, for example *PfKelch13* C580Y in Cambodia and *PfKelch13* F446I in upper Myanmar, and resistance appears to have emerged in multiple locations *(17, 18)*. In Africa, despite the presence of *PfKelch13* mutations, there is no evidence for selection of these mutations *(18, 19)*. This may be because additional permissive or compensatory mutations might be needed for the spread of *PfKelch13* mutations *(18)*. In Cambodia, the *PfKelch13* C580Y mutation has risen to dominance and spread across the country. A recent observation is that a single lineage of *PfKelch13* C580Y mutant parasites has spread across an area encompassing western Cambodia, north-eastern Thailand and southern Lao PDR. This implies a single origin of these apparently fit parasites (within the context of these GMS countries). Some of these emerging parasites have acquired the *Pfplasmepsin 2-3* marker for piperaquine resistance. In experimental models of transmission, Cambodian artemisinin-resistant clinical isolates were able to infect diverse mosquito vectors of South-East Asia and Africa, suggesting the potential for expansion of resistant parasites to regions outside the GMS *(20)*. In the GMS, *P. falciparum* resistant to artemisinin and/or partner drugs represents an emergency requiring the high-quality and urgent execution of the GMS malaria elimination agenda.

Conclusion

The slow parasite clearance phenotype that emerged and was selected following artemisinin treatment resulted in multiple soft sweeps of various *PfKelch13* mutations. These appear to have been overtaken by a hard sweep of the *PfKelch13* C580Y mutation, with a single dominant haplotype spreading across a wide geographic area involving three countries in the central part of the GMS. In the GMS, the presence of artemisinin-resistant *P. falciparum* malaria, which is increasingly compounded by partner drug resistance, is an emergency and should be treated as such. It requires the rapid, high-quality execution of the malaria elimination agenda in the GMS with a genuine sense of urgency.

Discussion

Although piperaquine-resistant strains are generally susceptible to mefloquine, mefloquine can select for resistance very rapidly. In the triple therapy trial (dihydroartemisinin-piperaquine-mefloquine), there have so far been no reports of resistance to all three agents. Monitoring studies of artesunate-mefloquine efficacy in Cambodia indicate 100% treatment efficacy, except in Ratanakiri, where efficacy is around 95%. Of concern is that in 250 samples in 2015, around 2.5% (six cases) had increased copy numbers for both *Pfmdr1* and *Pfplasmepsin 2-3*. There is no hard evidence that these parasites cannot achieve resistance to both mefloquine and piperaquine (as well as to the artemisinins).

Unlike in Cambodia, in Africa there has been no significant drug pressure from piperaquine, which may explain why indigenous resistant haplotypes have not yet spread, or why imported resistant parasites have not become established. Selection under increasing use of dihydroartemisinin-piperaquine could lead to the spread of these Asian parasites to Africa or the emergence of locally generated resistant strains.

Worldwide situation of drug efficacy and drug resistance outside the GMS (P. Ringwald)

More than 200 non-synonymous mutations in the *PfKelch13* propeller region have been reported worldwide. However, only *PfKelch13* N458Y, Y493H, R539T, I543T, R561H and C580Y have been validated through RSA as contributing to artemisinin resistance, while *PfKelch13* A578S and E252Q have been confirmed as not associated in vitro with artemisinin resistance. The impact of other *PfKelch13* propeller mutations on artemisinin resistance is still unknown.

The *PfKelch13* C580Y mutation, which has become dominant in western Cambodia, has also been identified in Viet Nam, Thailand, Lao PDR and Myanmar. Although this mutation has been detected sporadically outside the GMS, including in Africa, there is no evidence of expansion elsewhere. A possible exception is Guyana (see below).

Despite the high proportion of parasites harbouring artemisinin resistance and delaying clearance, the actual number of parasites on day 3 is low. Data from Cambodia show that patients treated with an ACT on day 3 have a parasitaemia representing 1–3% of initial parasitaemia (day 0). There is currently no evidence of the emergence of high-level artemisinin resistance.

India: *PfKelch13* mutations are rare and heterogeneous, and do not lead to treatment failure. In northern India, where the risk of importation of artemisinin-resistant haplotypes from the GMS is highest, surveillance has infrequently identified *PfKelch13* mutations; to date, no C580Y has been detected. However, artesunate-sulfadoxine-pyrimethamine treatment failures have occurred in India because of a shift from double and triple *Pfdhfr* and *Pfdhps* mutants to quadruple and quintuple mutants, respectively. These parasites have also been observed in Somalia and Sudan, but are still rare in Afghanistan, the Islamic Republic of Iran, and Pakistan.

Africa: Non-synonymous *PfKelch13* mutations are still rare and highly diverse in Africa *(16)*. The most frequent allele observed in Africa is A578S, which is not associated with clinical or in vitro artemisinin resistance. Those mutations that are known to be associated with artemisinin resistance have not expanded in African parasite populations.

Amodiaquine resistance and sulfadoxine-pyrimethamine resistance emerged in Africa before the introduction of ACTs. ACT efficacy across Africa remains generally high. Artemether-lumefantrine efficacy is >90%, although investigation into the possible presence of lumefantrine resistance in Angola continues. Artesunate-amodiaquine is used in areas where amodiaquine remains efficacious. The two remaining countries using artesunate-sulfadoxine-pyrimethamine (Somalia and Sudan) will cease treatment with this combination in the near future due to sulfadoxine-pyrimethamine resistance. For dihydroartemisinin-piperaquine, increased *Pfplasmepsin 2-3* copy number has been detected infrequently, although these cases have yet to be confirmed. If confirmed, this indicates the potential for piperaquine resistance to emerge and spread in Africa.

South America: In Guyana, a retrospective analysis of blood samples collected in 2010 for a *Pfhrp2* surveillance study detected five samples with *PfKelch13* C580Y. All five samples had nearly identical haplotypes, suggesting a common origin, distinct from the South-East Asian *PfKelch13* C580Y haplotype. In 2014, a 7-day trial of artesunate 4 mg/kg/day + primaquine (*n* = 50) resulted in a 2% day-3 positivity rate and 100% efficacy; all samples were *PfKelch13* wild-type. A survey conducted in 2016 identified *PfKelch13* C580Y at a frequency of 3.3%, although this frequency was 11.9% in one sample area. As far as is known, ACT efficacy has not been affected, although there are no clinical data for the parasites identified with *PfKelch13* C580Y. Quality control and flanking microsatellite profiles are ongoing. This case may, therefore, represent an independent emergence and limited spread of a *PfKelch13* C580Y haplotype unrelated to that observed in South-East Asia. The proportion of parasites with increased *Pfplasmepsin 2-3* copy number in South America is currently unknown.

Conclusion

Outside the GMS, there is little evidence of artemisinin resistance or the proliferation of *PfKelch13* mutant parasites, except perhaps in Guyana.

Discussion

Although it is clear that the *PfKelch13* C580Y mutation detected in Guyana in 2010 is not an Asian parasite, it is not yet known whether the *PfKelch13* C580Y mutation detected in 2016 represents a single haplotype or whether this mutation arose several times independently on different background haplotypes.

Studies to further characterize the functional impact of *PfKelch13* mutations are ongoing. However, there are many different mutations to be tested. Gene-editing and transfection studies are challenging, although there is a new laboratory parasite that may be more amenable to transfection. Knockout studies may provide enough information to determine whether a mutation has functional relevance to artemisinin resistance.

If artemisinin resistance were to extend from the ring stage to other life cycle stages, it would be evident with increasing IC_{50} values using traditional in vitro assays. A trend of increasing artemisinin IC_{50} values has been observed in Cambodia, but with high variation and a large degree of overlap between resistant and sensitive parasites. A 7-day artemisinin trial can be used to investigate whether artemisinin resistance can cause delayed clearance and clinical failure. So far, there have only been four cases where artemisinin resistance has led directly to both.

Drug development landscape (T. Wells)

In the GMS, it may be necessary to move to triple combination therapy in order to sustain efficacy in some regions. In Africa, the emergence of dihydroartemisinin-piperaquine treatment failure may be related to the poor stability of the drugs, as non GMP-certified agents are available in this region.

For new combination therapies *(21)*.

- OZ439/ferroquine: There is a risk of cross-resistance between ferroquine and piperaquine/chloroquine/amodiaquine, although no strong correlation has been found in vitro. To date, resistance to OZ439 has not been induced in the laboratory. However, parasites harbouring a *PfKelch13* mutation exhibit reduced sensitivity to OZ439, although this is mitigated by a longer half-life than for dihydroartemisinin. This should result in the significantly improved efficacy of OZ439 against *PfKelch13* mutant parasites *(22)*.
- KAF156/lumefantrine: This combination is being developed as a single-exposure cure and prophylaxis agent, and as a 3-day treatment for multidrug-resistant parasites. Phase II will be completed in 2018. This combination is susceptible to pre-existing lumefantrine resistance. KAF156 has a mild resistance risk evaluated in vitro.
- In addition, KAE609 (cipargamin), DSM265, MMV048 and SJ733 are in human studies.

Thus, it is possible to develop combinations with two novel drugs or even triple therapies. In the absence of partner drug resistance, treatment efficacy rates with existing ACTs remain high in most regions. Demonstrating the efficacy of new combinations requires larger studies with non-inferiority endpoints. Following this conventional path, the earliest registration for a new combination therapy is anticipated in 2022–2023. However, should drug-resistant malaria be prevalent enough to conduct clinical trials, conditional approval might be possible based on Phase IIb data, thereby accelerating registration. The current low number of clinical malaria cases with ACT resistance and their occurrence in countries not amenable to clinical research does not facilitate large-scale clinical trials. Therefore, it is important to preserve the efficacy of currently available agents and investigate novel deployment strategies.

Situation in Cambodia (D. Kwiatkowski)

There are multiple origins of artemisinin resistance in Cambodia, but *PfKelch13* C580Y is spreading faster than other *PfKelch13* mutations. Although there are several haplotypes for *PfKelch13* C580Y, one origin accounts for more than half of all *PfKelch13* mutations in Cambodia. This dominant haplotype has spread to multiple locations and has recombined onto other genetic backgrounds. In western Cambodia, C580Y is approaching fixation.

Pfplasmepsin 2-3 amplification has been observed mainly, but not exclusively, in parasites that have the *PfKelch13* C580Y mutation. *Pfplasmepsin 2-3* amplification is a strong marker for dihydroartemisinin-piperaquine treatment failure. In western Cambodia, the proportion of parasites with multiple *Pfplasmepsin 2-3* copy number increased from 0% in 2007 to around 75% in 2013. *Pfplasmepsin 2-3* copy numbers have also increased from double to triple to quadruple. In the same dataset, the proportion with *PfKelch13* mutations rose from 20% in 2007 to around 90% from 2008 to 2013. By contrast, the proportion of parasites with multiple *Pfmdr1* copy number decreased from around 55% in 2007 to 5% in 2013.

Pfplasmepsin 2-3 amplifications in western, northern and north-eastern Cambodia have a single main origin, as indicated by the same haplotypes and breakpoints. However, across a wider area, multiple origins are probable and have been detected in other datasets from Cambodia. *Pfplasmepsin 2-3* amplifications are spreading across Cambodia and increasing in copy number over time. In northern Cambodia, parasites without *Pfplasmepsin 2-3* amplification have mainly north-Cambodian ancestry, whereas those with amplifications have mainly west-Cambodian ancestry. This indicates the invasion of resistant parasites that are replacing the indigenous piperaquine-susceptible parasites. Further studies are examining samples from Viet Nam. Although there may be additional resistance mechanisms, coincident *PfKelch13* C580Y and *Pfplasmepsin 2-3* amplification have been found to cause high rates of dihydroartemisinin-piperaquine treatment failure (>25%). This selective sweep is spreading very rapidly across Cambodia, most likely due to extensive drug pressure.

Independent emergence and spread of artemisinin resistance (C. Plowe)

In the GMS, haplotype network analysis has indicated that different *PfKelch13* mutations have arisen independently on many different genetic backgrounds. Some mutations have independently emerged ('popped') and not spread, whereas others have emerged and spread ('jumped') between different sites. The *PfKelch13* C580Y mutation has both emerged independently multiple times and spread transnationally multiple times, including one instance where a lineage spread from the southern tip of the Myanmar peninsular to Cambodia, traversing the non-malarial region of Thailand *(23)*. The *PfKelch13* F446I mutation has similarly spread over a large area along the China–Myanmar border and in northern and north-eastern Myanmar, areas where the *PfKelch13* C580Y mutation remains rare. These examples suggest that there have been multiple instances of different mutations spreading widely on different genetic backgrounds. This does not preclude the possibility that a single highly resistant parasite haplotype could become dominant throughout the GMS and spread outside the GMS, but to date the patterns of emergence and spread are complex and geographically variable.

There is evidence of other loci rising to high frequency in the parasite population, and these may have enabling or compensatory functions with regard to artemisinin resistance. Notably, a minority of parasites have an artemisinin-resistant phenotype (delayed parasite clearance), but no mutations in *PfKelch13*. Conversely, not all *PfKelch13* mutations confer the artemisinin-resistant phenotype, suggesting that additional genetic changes may be necessary to achieve resistance.

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ERG on multidrug-resistant *P. falciparum* in the GMS

Minutes of ERG meeting
Presented by D. Wirth, Chair of the ERG

Geneva, 22-24 March 2017
MPAC meeting

Global **Malaria** Programme

World Health Organization

Background

- At the Malaria Policy Advisory Committee (MPAC) meeting, 14–16 September 2016, Professor N. White (Mahidol Oxford Tropical Medicine Research Unit) cited new evidence of a multidrug-resistant *P. falciparum* parasite lineage;
- MPAC requested that an Evidence Review Group (ERG) assess the relevance of the information and report the potential implications to MPAC at the next meeting in March 2017.
- Meeting was organized 20-21 December in Geneva

Situation of drug resistance in the greater Mekong

- Artemisinin resistance in *P. falciparum* has emerged independently and evolved in the GMS over the past decade;
- Even in the presence of artemisinin resistance, efficacy rates of ACTs may still be adequate. It is only once resistance to the partner drug emerges that treatment failure rates increase significantly;
- Resistance to artemisinin-based combination therapy (ACT) partner drugs, including piperaquine and mefloquine, has been detected in the GMS resulting in the declining efficacy of some of the recommended ACTs;
- In response to the drug resistance situation and the declining number of malaria cases, in May 2015, GMS Ministers of Health adopted the *Strategy for malaria elimination in the Greater Mekong subregion 2015–2030*.

Objectives of the meeting

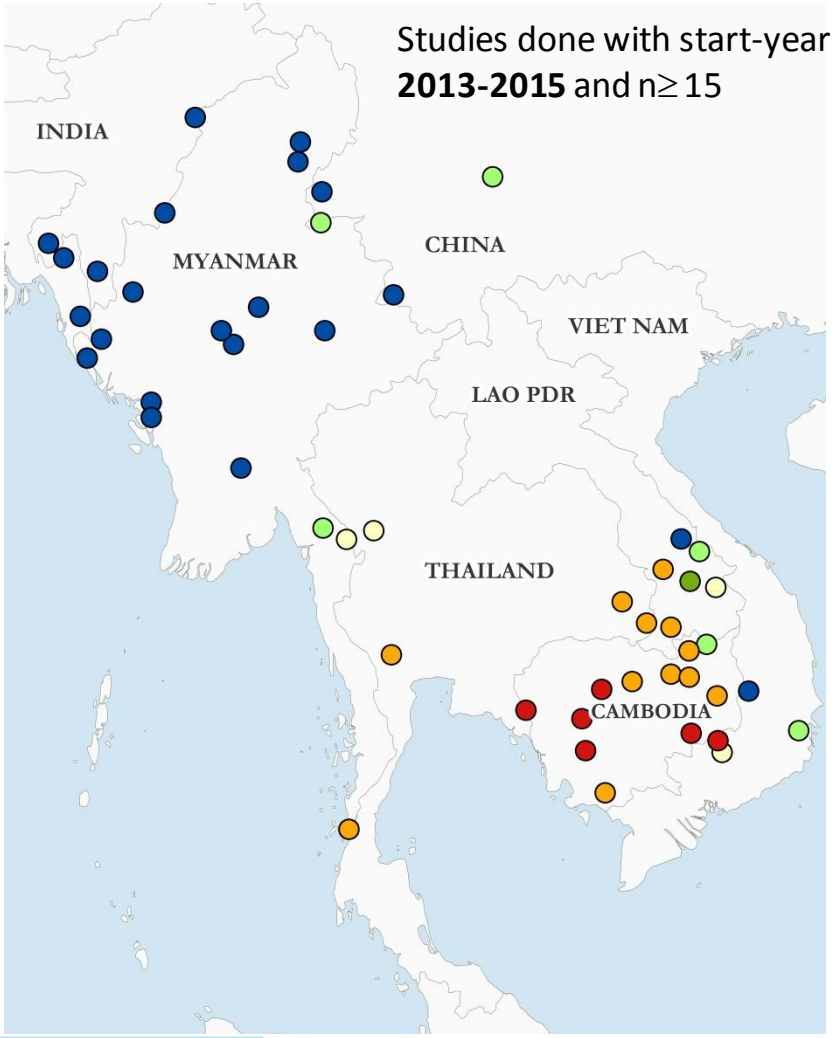
General Objective

- To discuss the emergence and spread of multidrug-resistant *P. falciparum* lineages in the GMS.

Specific Objectives

- To review new evidence on the emergence and spread of multidrug-resistant *P. falciparum* lineages with the *PfKelch13* C580Y mutation and the *Pfplasmepsin* 2-3 gene amplification in the GMS;
- To assess the risk posed by these parasites in terms of malaria control and elimination in the GMS and in other parts of the world;
- To identify evidence gaps and provide recommendations for further research.

Percentage of samples with C580Y mutation



Percentage of parasites with C580Y

- 0
- 0.01 - 5
- 5.01 - 20
- 20.01 - 50
- 50.01 - 90
- 90.01 - 100

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Process and presentation

- Three manuscripts were shared and presented by the relevant research groups:
  - Anderson TJ, Nair S, McDew-White M, Cheeseman IH, Nkhoma S, Bilgic F, et al. Population parameters underlying an ongoing soft sweep in Southeast Asian malaria parasites. Mol Biol Evol. 2017;34(1):131–44
  - Cerqueira G, Cheeseman I, Schaffner S, Nair S, McDew-White M, Pyae Phyo A, et al. Longitudinal genomic surveillance of Plasmodium falciparum malaria parasites reveals complex genomic architecture of emerging artemisinin resistance in western Thailand. 2016 (http://biorxiv.org/content/early/2016/11/02/084897).
  - Imwong M, Suwannasin K, Kunasol, C, Sutawong K, Mayxay, M, et al. A molecular epidemiology observational study of the recent transnational spread of artemisinin resistant P. falciparum in the Greater Mekong Subregion. Lancet Infect Dis 2017 (in press).
- The ERG panel addressed the following key questions:

Question and answers

Is there new evidence of selection and spread of specific artemisinin-resistant genotype(s) in the GMS?

- *PfKelch13* C580Y mutation can be found in several genetic backgrounds (at least 3 haplotypes) throughout the GMS;
- The prevalence of one specific *PfKelch13* C580Y haplotype is increasing, replacing other haplotypes in an area that includes sites in western Cambodia, north-eastern Thailand and southern Lao PDR;
- This lineage is also resistant to piperaquine in western Cambodia and north-eastern Thailand;
- However, the frequencies of different *PfKelch13* C580Y haplotypes vary by region, and no single haplotype is dominant throughout the GMS.

Question and answers

What would be the consequences of the selection and spread of specific artemisinin-resistant genotype(s)?

- Partial or total loss of efficacy with respect to artemisinin treatments;
- Global spread of a dominant haplotype encoding that would increase levels of resistance;
- A common genetic background could accumulate mutations that might encode potential compensatory factors, such as ACT partner drug resistance, fitness or transmissibility;
- Alternatively, the spread of *PfKelch13* C580Y haplotypes could result in a loss of within-population genetic diversity (fitness disadvantage).

Question and answers

Is there evidence that artemisinin resistance has facilitated the emergence of partner drug resistance in the GMS?

- There is evidence of parasites with resistance to both artemisinin and piperaquine in Cambodia and areas of Thailand bordering Cambodia;
- Piperaquine resistance has occurred in the past in China and Cambodia independently of artemisinin resistance;
- Therefore, artemisinin resistance is not a prerequisite for the initial appearance of piperaquine resistance;
- It is not possible to determine the temporal relationship between the emergence of either resistance;
- There is no evidence that *PfKelch13* C580Y confers any resistance to piperaquine.

Question and answers

Is there evidence that artemisinin resistance has facilitated the selection and spread of partner drug resistance in the GMS?

- As the resistance mechanism is not understood, it is difficult to understand the spread of resistance in the different genetic backgrounds;
- Different factors affect the selection and spread of piperaquine resistance:
  - Artemisinin resistance may increase the exposure of the parasite population to piperaquine;
  - Piperaquine long half-life (present as a monotherapy for about 1 month);
  - Conversely, it is possible that the reduced efficacy of piperaquine has facilitated the selection of artemisinin-resistant mutations.
- Not enough data are available to draw any conclusions for other ACTs except that there was a background of mefloquine resistance in the GMS prior to the introduction of artemisinin drugs.

Question and answers

Is there evidence of the geographical extent of artemisinin resistance outside the GMS?

- There is evidence of multiple *PfKelch13* mutations in many geographic regions:
  - none of these are associated with a haplotypic expansion of *PfKelch13* C580Y;
  - no evidence to indicate artemisinin resistance outside the GMS (according to WHO definition);
- One exception is the possible independent emergence of *PfKelch13* C580Y in Guyana.

Question and answers

What is the risk of the spontaneous emergence, selection and spread of artemisinin resistance and/or resistance to ACT partner drugs outside the GMS? (1)

- Historically, chloroquine, sulfadoxine and pyrimethamine resistance emerged in South-East Asia and spread.
- Mefloquine resistance also emerged in the GMS, but it has not spread to other regions;
- PfKelch13 mutations and probably piperaquine-resistant parasites are present at low frequencies in P. falciparum outside the GMS including Africa and elsewhere;

Question and answers

What is the risk of the spontaneous emergence, selection and spread of artemisinin resistance and/or resistance to ACT partner drugs outside the GMS? (2)

- There is the potential that these variants may rapidly select and spread:
  - The massive and uncontrolled use of dihydroartemisinin-piperaquine in settings outside the GMS (Africa) may lead to resistance and loss of efficacy of this treatment;
  - The fact that the PfKelch13 C580Y mutation is not spreading suggests that additional mutations may be necessary; under drug pressure, however, the necessary compensatory mutations might be acquired;
- Overall, there is a significant risk of artemisinin and partner drug resistance outside the GMS – either via spontaneous emergence or importation, and spread.

Question and answers

Identify research questions that might improve our understanding of artemisinin resistance

- Biologically characterize artemisinin resistance mutations and mechanisms of artemisinin resistance;
- Investigate the impact of parasite population characteristics, such as population genetic structure, on the emergence and spread of drug resistance;
- Assess the role of human mobility and drug use in the emergence and spread of resistance;
- Explore alternative drug regimens;
- Determine the contribution of artemisinin resistance to the spread of multidrug-resistant malaria parasites;
- Investigate the contribution of partner drug resistance to the spread of multidrug-resistant parasites.

Conclusions (1)

- Independent emergence and transnational spread of different lineages of artemisinin-resistant parasites have occurred throughout the Greater Mekong subregion (GMS).
- These multidrug-resistant parasites have been responsible for increasing dihydroartemisinin-piperaquine failure rates across Cambodia over the last 5 years.
- Artesunate-mefloquine is currently efficacious in Cambodia, with cure rates >95%, and is being used as first-line treatment in Cambodia as an intermediate solution.

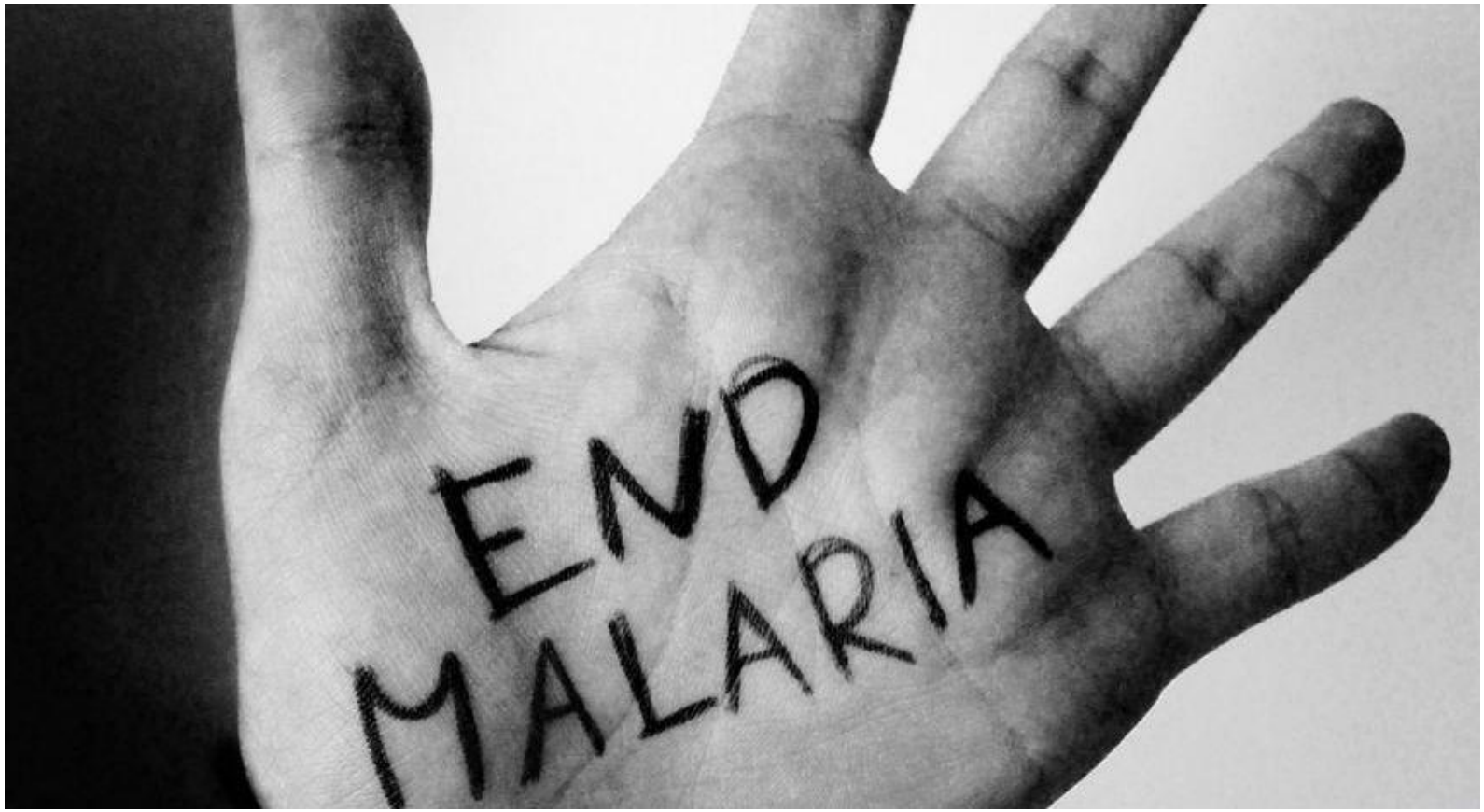
Conclusions (2)

- The risk of a highly fit multidrug-resistant lineage spreading widely in higher transmission settings cannot be discounted.
- This risk is mitigated by the likelihood that resistance and/or fitness mutations residing on different chromosomes would be rapidly broken up by recombination in multiclonal infections.
- Similar to the situation in South-East Asia, changing transmission dynamics in Africa have resulted in larger regions of lower malaria transmission intensity.
- The shift in Africa towards higher drug pressure and less outcrossing in Africa increases the potential for the selection and spread of locally generated resistant strains.
- There is also a possibility for parasites from GMS to potentially become established and spread following importation. Nevertheless, issues of fitness, genetic complexity of the multi-resistant parasites and reduced prevalence of malaria in the GMS mitigate this risk.

Recommendations

- The new data reaffirm the need for an urgent and continued intensive regional malaria elimination campaign in the GMS;
- Surveillance for artemisinin and partner drug resistance needs to be continued and strengthened in the GMS;
- There is a critical need for surveillance outside the GMS to detect potential de novo resistance or the potential 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.

Thank you for your attention



Malaria Policy Advisory Committee Meeting
22–24 March 2017, Geneva, Switzerland
Background document for Session 7

World Health Organization

P. falciparum hrp2/3 gene deletions

Situation Update, Geneva, Switzerland

Background

Since May 2016, the WHO Global Malaria Programme (GMP) has published and updated an information note for the manufacturers, procurers and users of HRP2-based RDTs with interim guidance on how to investigate suspected false-negative RDT results, including *pfhrp2.3* gene deletions, and on alternative non-HRP2-based RDT options (http://www.who.int/malaria/publications/atoz/information-note-hrp2-based-rdt/en/).

In parallel, a technical consultation on *P. falciparum hrp2/3* gene deletions was held in Geneva on 7–8 July 2016. The final conclusions and recommendations from this consultation were presented to the Malaria Policy Advisory Committee (MPAC) in September 2016 (http://www.who.int/malaria/mpac/mpac-sept2016-hrp2-consultation-short-report-session7.pdf).

This briefing paper provides an update on the current situation and planned next steps, based on the recommendations that emerged from the technical consultation and those received by MPAC at its last meeting (http://www.who.int/malaria/publications/atoz/mpac-september2016-report.pdf).

i) WHO should promote a harmonized approach to investigating, surveying and reporting *pfhrp2/3* gene deletions through the provision of standard protocols (including sample size calculations) and operating procedures.

A standard protocol for estimating the prevalence of *pfhrp2/3*-deleted parasites (at province level) across geographical areas is in the final stages of revision and expected to be available in April 2017. The protocol will guide surveys estimating the prevalence of *pfhrp2/3* gene deletions among symptomatic patients presenting to health facilities. This protocol will include a list of international reference laboratories and their respective capacities to support investigations. In addition, a set of external quality assessment (EQA) materials including *pfhrp2/3* gene deletions will be prepared to support proficiency testing at these reference laboratories. Such materials will ensure reliability and comparability between laboratories. Run controls will also be made available for PCR quality control (see also point vi, below).

ii) Generally, *pfhrp2/3* surveys and surveillance activities should first target countries where deletions or concerns have been identified, and the neighbouring countries.

WHO/GMP is actively supporting the design and planning of surveys for *pfhrp2/3* gene deletions in the states/provinces of Ethiopia and Sudan bordering Eritrea. The surveys are expected to take place during the high-transmission season (September/October 2017). Sampling will be powered in order to obtain precise estimates of pfhrp2/3 gene deletions at the province level above or below the 5% threshold.

WHO/HTM/GMP/MPAC/2017.6

iii) WHO should integrate information about *pfhrp2/3* gene deletions into the global mapping database currently under development.

The online global mapping database for insecticide and drug resistance has been adapted to accommodate reporting of *pfhrp2/3* gene deletions (both positive and negative findings). A review was conducted of all published (and some unpublished) reports of *pfhrp2/3* gene deletions, and data were extracted to inform the online global mapping database. The review yielded data from 20 countries and 110 distinct datasets.

Since the last MPAC meeting, new reports of *pfhrp2/3* gene deletions have emerged from Rwanda, Uganda, Bangladesh and Mozambique.

iv) The published recommended procedures for investigating and accurately reporting *pfhrp2/3* deletions are comprised of three steps: establishing initial evidence, establishing confirmatory evidence, and establishing prevalence (Cheng Q et al., *Malaria Journal* 2014 13:283). The methodology proposed in this paper should be revised to recommend that confirmatory evidence include PCR for *pfhrp3* in addition to PCR for *pfhrp2*, as HRP3 proteins can show cross-reactivity in HRP2-based RDTs; however, the analysis of flanking genes for *pfhrp2* (and *pfhrp3*) and the confirmation of absent HRP2 antigen (by ELISA or second brand of RDT) are optional.

The WHO information note has been updated to reflect these changes.

v) *Pfhrp2/3* gene deletions pose an urgent public health threat, but they are challenging to confirm. Therefore, to promptly and effectively respond to the threat, WHO should establish a consortium to provide technical support in investigating suspected false-negative RDTs due to *pfhrp2/3* deletions, to establish appropriate surveillance systems, and to elaborate on the factors influencing the emergence and spread of *pfhrp2/3* deletions.

WHO/GMP has established a network of reference laboratories that will support investigations into suspected false-negative RDTs due to *pfhrp2/3* gene deletions. More resources will be required to expand this network to include surveillance systems and the underlying environmental and biological factors driving the emergence and spread of *pfhrp2/3* gene deletions.

As part of these harmonization efforts, during the ASTMH annual meeting, WHO/GMP co-organized a side-meeting on "*Pfhrp2/3* gene deletions: Update, implications and response". Reports of *pfhrp2* gene deletions from various regions of the world were summarized, and models of RDT selection pressure and spread in Africa, policy implications and future directions were presented and discussed.

vi) Tests with both HRP2 and pLDH antibodies on the same test line should be prioritized for assessment by WHO prequalification, including a laboratory evaluation against *pfhrp2/3* single- and double-deleted parasites (culture and clinical samples) to determine whether the tests meet recommended performance criteria. Programmes should not replace Pf-only HRP2-based RDTs with current HRP2/pan-pLDH or aldolase combination tests for the purpose of detecting non-HRP2-expressing parasites; only RDTs that specifically target pf-pLDH or pan-pLDH-only tests should be used.

WHO/GMP identified sources of culture-adapted isolates of *pfhrp2*-negative parasites in a small collection of archived wild-type HRP2-negative samples (200, 2000p/µl pairs) from Peru. With support from FIND, it initiated the prospective collection of *pfhrp2*-negative *P. falciparum* parasites in Peru (Universidad Peruana de Cayetano Heredia), which is now ongoing. These materials will be characterized and selected for inclusion in Round 8 of the WHO malaria RDT product testing panels, starting in March 2017. Round 8 is comprised of 35 RDTs and includes 10 products that target non-HRP2 antigens for detection of *P. falciparum*.

vii) Develop a plan of action for surveillance and response that can be supported by partners and implemented in countries.

An outline for a plan of action and response has been drafted, and an expert in infectious diseases and diagnostics who is familiar with the complexity and details of global malaria diagnostics, molecular biology, protein-ligand interactions, assay development, and HRP2 detection has been contracted to draft the document over the next 2 months. The contents are described below:

Executive summary

Problem statement

- History of RDT use and its current role in disease control and patient management (include types of tests, usage volumes and brief discussion of quality management mechanisms in place);
- Role of RDTs in achieving the goals of the *Global technical strategy 2016–2030*;
- Identification of HRP deletion mutants (history of our knowledge and evidence of the current size of the problem) [make point that not enough is known about the extent of the spread and clinical impact to take definitive action at present, but there must be a plan];
- Impact of RDT failure on tracking malaria incidence (versus other background diagnostic failures);
- Clinical and disease-control impact to date – examples from countries on how they are managing.

State of knowledge and research gaps

- Understanding the genetics of deletion (mechanism, genetic variability, drivers, fitness);
- Understanding the temporal origin of HRP deletion and mechanism of spread;
- Understanding technical factors that affect HRP2 detection (expression of HRP3, parasite density, circulating HRP2 antibodies, make of RDT and specific monoclonal used);
- Understanding the global epidemiology of deletions – the current size of the problem and the clinical impact;

- Modelling the future (with and without mitigation measures);
- Current status of pLDH-based RDTs and the hope for better RDTs (short- and medium-term): alternative falciparum markers that are abundant and species-specific.

Surveillance plan

- Clarifying the current size of the problem and its impact:
    - Strengthening laboratory networks (national and supranational) for surveillance and monitoring (standardized methods for sampling, testing, data management);
    - Options for mapping prevalence, i.e., which sites/countries/epidemiologic situations should be prioritized.
- Temporal and spatial tracking of HRP deletion prevalence:
    - Options for tracking over time (compare with other efforts in the longitudinal tracking of mutant types and mutation rates (e.g., AMR), lay out choices, costs and usefulness of different sampling methods);
    - Establishing triggers for action (prevalence cut-offs, outbreak sizes, trend speed).

Managing response

- Case detection and case management strategies at trigger points (dual testing, etc.);
- Risk communication with countries/national programmes;
- Engagement with the diagnostics industry;
- Procurers (cost constraints, complexity of procuring >1 RDT type and full product replacement);
- Changes required to WHO Product Testing;
- Interaction with regulatory/qualifying bodies.

Resource requirements to respond to the threat of *pfhrp2*-deleted parasites

vii) Resource mobilization

The response plan will be utilized to mobilize resources to support the required actions. In the interim, WHO/GMP is investing a contribution from Bill & Melinda Gates Foundation (US$ 150 000) to support the following:

1. To develop a plan of action for surveillance and response to the emergence and spread of *pfhrp2/3* gene deletions;
2. To develop a) standard protocols (and tools) for conducting baseline surveys and surveillance for the prevalence of *pfhrp2/3* gene deletions, and b) report templates;

3. To establish a consortium of malaria reference laboratories and research institutes to provide methodologies and technical support and to conduct analyses for ruling in or excluding *pfhrp2/3* gene deletions. The outputs of the consortium will link with appropriate surveillance systems in order to map the emergence and spread of *pfhrp2/3* deletions;

4. To provide technical and financial support to countries investigating suspected *pfhrp2/3* gene deletions or implementing survey/surveillance activities. Priority will be given to countries at high risk due to their proximity to areas or countries with known high prevalence of *pfhrp2/3* gene deletions.

Update on *Plasmodium falciparum* hrp2/3 gene deletions

Jane Cunningham – MPAC 22–24 March 2017

Global **Malaria** Programme

World Health Organization

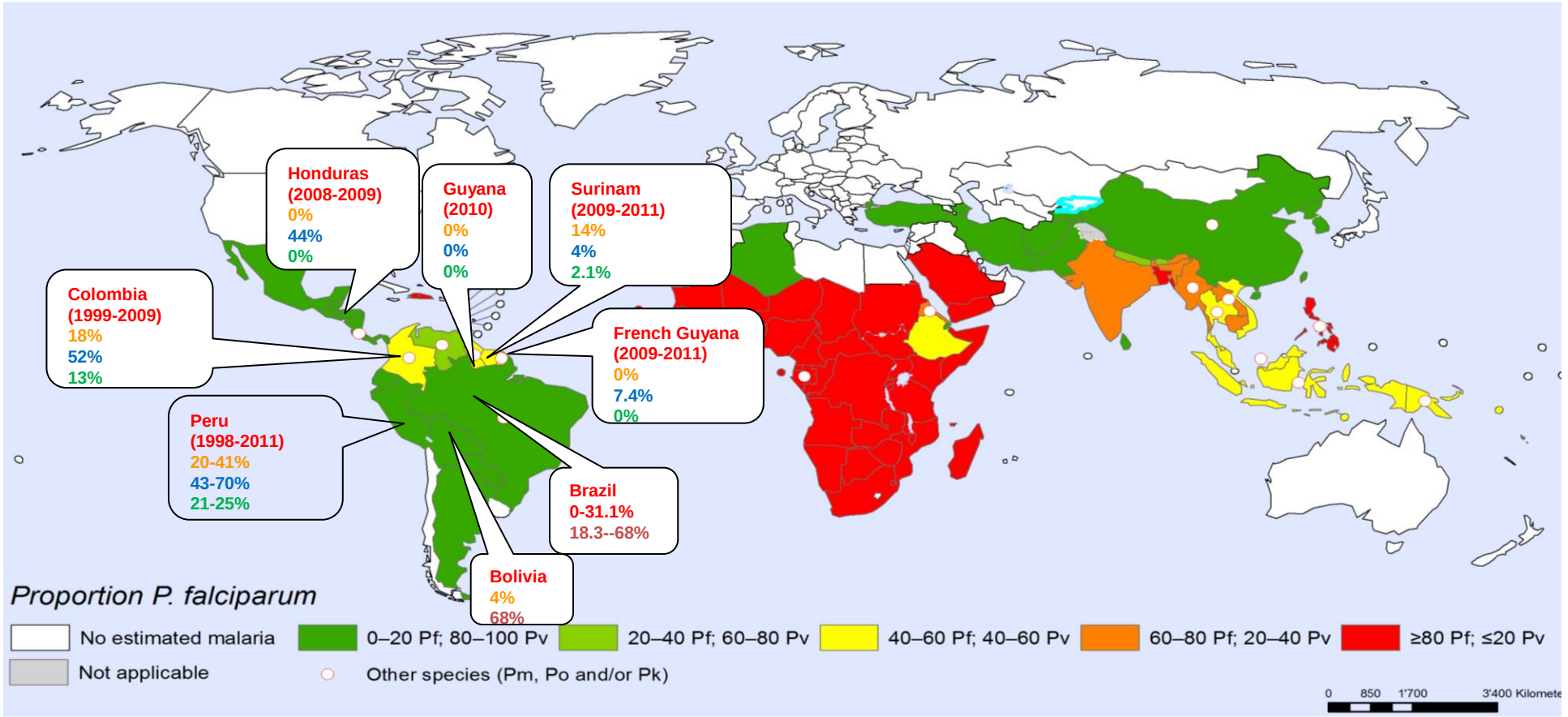
Overview

- Summary of reports: *pfhrp2/3* gene deletions reports: Central/South America, Africa, Asia

- Progress report on action items (1-7) from MPAC – Sept 2016

Central and South America

Parasites Lacking HRP2/3 in Central and South America



Gamboa et al 2010
Maltha et al 2012
Akinyi et al 2013
Houze et al 2011
Trouvay et al 2013
Akinyi et al 2015
Murillo et al 2015
Abdallah et al 2015
Dorado EJ et al 2016
Rachid Viana GM et al 2017

Global **Malaria** Programme

Slide courtesy of Q. Cheng, AMI

World Health Organization

Spatial heterogeneity: Brazil

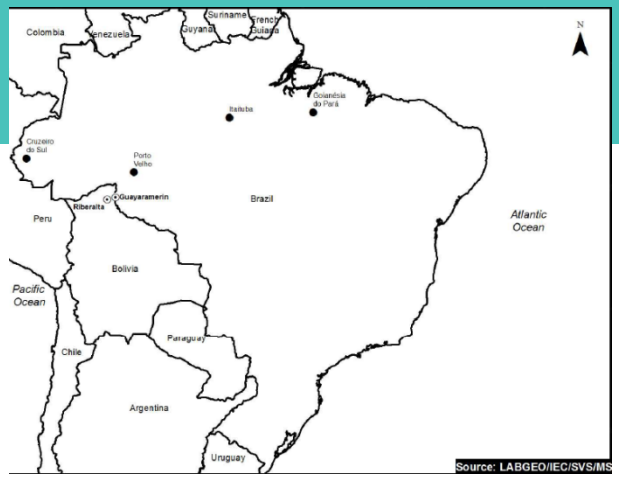


Table 1. ***Pfhrp2*** **and** ***pfhrp3*** **gene deletions among samples collected in Brazil (198 samples) and Bolivia (25 samples).**

Site	*Pfhrp2* deletion		*Pfhrp3* deletion		Total
	N (%)	95% confidence interval	N (%)	95% confidence interval	
Acre, Brazil	25/79 (31.6%)*	21.6–43.1	30/79 (38.0%)	27.3–49.6	79
Para, Brazil	0/59 (0%)	NA	30/59 (50.9%)	37.5–64.1	59
Rondonia, Brazil	2/60 (3.3%) *	0.4–11.5	11/60 (18.3%)	9.5–30.4	60
Bolivia	1/25 (4.0%)	0.1–20.4	17/25 (68.0%)	46.5–85.1	25

https://doi.org/10.1371/journal.pone.0171150.t001

* - a total of 23 double negative for *pfhrp2* and *pfhrp3* were detected in Acre (21 samples) and Rondonia (2 samples).

Rachid Viana GM, Akinyi Okoth S, Silva-Flannery L, Lima Barbosa DR, Macedo de Oliveira A, Goldman IF, et al. (2017) Histidine-rich protein 2 (*pfhrp2*) and *pfhrp3* gene deletions in *Plasmodium falciparum* isolates from select sites in Brazil and Bolivia. PLoS ONE 12(3): e0171150. https://doi.org/10.1371/journal.pone.0171150

Africa

Pfhrp2/3 deletion reports

Published: Mali (2012)

Senegal (2013)

Ghana (2016)

DRC (2016)

Rwanda (2017)

Unpublished (2016): Eritrea (pre-submission)

Mozambique (submitted)

Zambia

Uganda

Reports: West Africa

Pfhrp2 PCR-negative reports:

Mali (Koita et al. AJTMH 2012), first African report

- **2.1%** (n=10) of 480 asymptomatic, micro-positive subjects

Senegal (Wurtz et al. Malar J 2013)

- **2.2%** (n=3) of 136 symptomatic, micro-positive subjects
- 12.8% *pfhrp3* negative

Ghana (Amoah et al. Malar J 2016)

- 124 asymptomatic *pfhrp2*-negative subjects

Reports: Southern Africa

Pfhrp2 PCR-negative reports:

Zambia (unpublished)- 2009-2013

- Community based surveys- Choma and Nchelenge
- 8 asymptomatic, RDT -/PCR+ subjects
- 0-4.7%

Mozambique (submitted for pub) – 2010-2016

- Cross sectional community survey – n=9124
- Pfhrp2/3 gene analysis for RDT-/micro+ or PCR + - n=164; many samples excluded due to poor quality DNA
- 69 samples analyzed - 1 asymptomatic subject had *pfhrp2* deletion

Reports: Eastern Africa*

New

Uganda (unpublished – **Nsobya ASTMH talk #1261**)

- **Household survey 2012-2013**
- **1.6% (n=25) of 1493 smear-pos/PCR-pos subjects** were *pfhrp2* PCR-negative
  - Of 96 RDT-neg/microscopy-pos subjects, only 56/96 (58%) confirmed PCR + : of these 56 : 25 (45%) *pfhrp2* PCR-negative, 39 (70%) *pfhrp3* PCR-negative, 19 (34%) had double deletions
- 3 sites: MOI 1.0-2.0, mean 225-700p/uL, EIR 3.8-125
- 44/56 samples with deletions were from Tororo

Rwanda (Kozycki et al. Malar J (2017) 16:123)

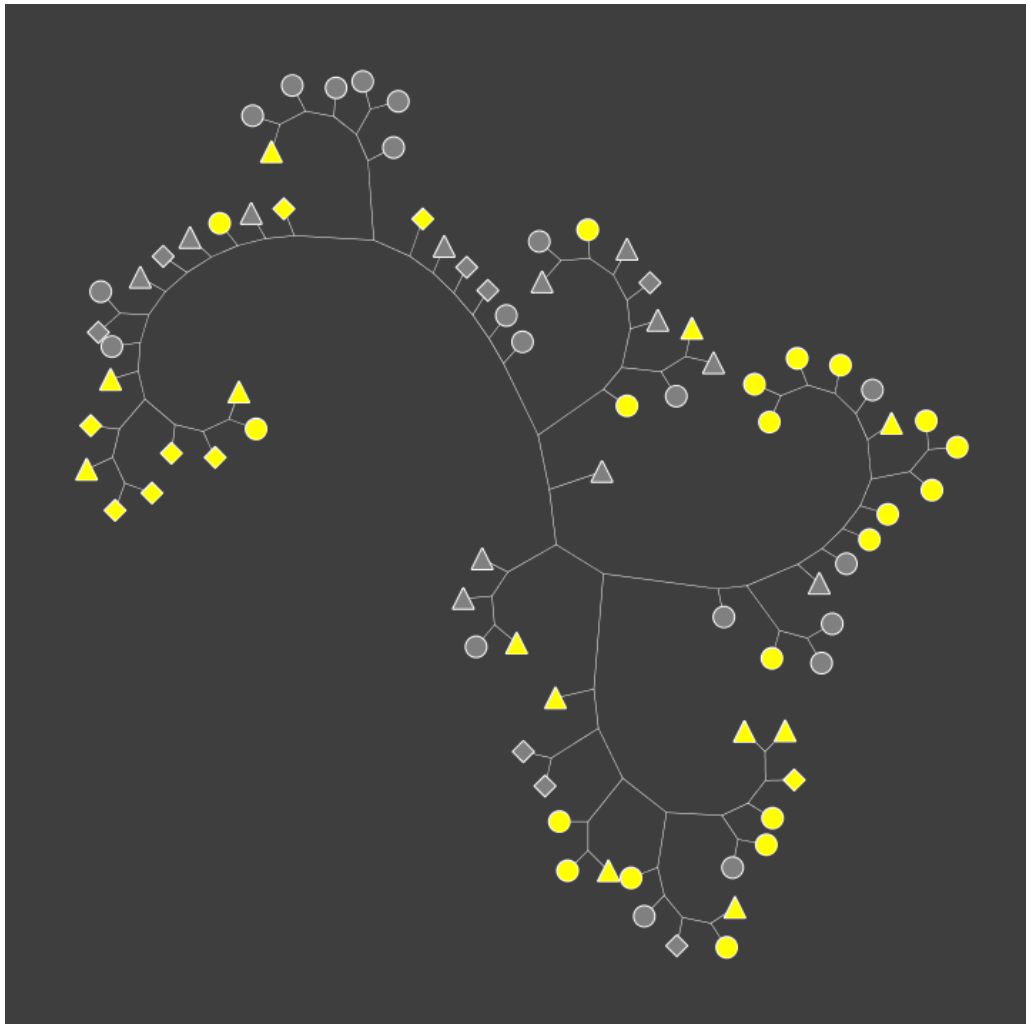
- DHS 2014-2015
- **1.0% (n=32) of 3291 smear-pos subjects** were *pfhrp2* PCR-negative
  - Of 322 were RDT-neg/PCR-pos, 32 *pfhrp2* PCR-negative
- 3 sites: EIR <1-21, slide positivity 0-4.4% in children

(* excluding Eritrea)

Reports: Central Africa

DRC (Parr et al. J. Inf Dis. 2016)

- **DHS survey**
- **6.4% (n=149) prevalence** among asymptomatic, PCR-pos subjects had a *pfhrp2* deletion.
  - Only 5 (3.4%) of these 149 also had a *pfhrp3* deletion
- **First national survey**
  - Deletions more common in areas of low malaria prevalence
- **Population genetics**
  - Deleted parasites are genetically distinct from controls



Reports: Eritrea

Eritrea (unpublished – **Berhane ASTMH poster #879**)

pfhrp2/pfhrp3 deletions in Eritrea - 2016

Rapid diagnostic tests failing to detect *Plasmodium falciparum* infections in Eritrea: an investigation of reported false negative RDT results

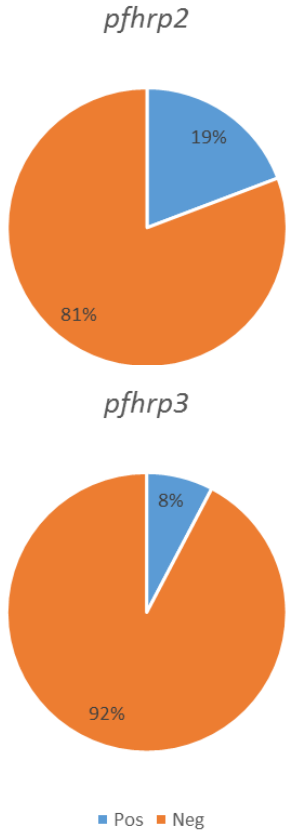
Araia Berhane[1], Mulugeta Russom[2], Iyassu Bahta[3], Filmon Hagos[4], Michael Ghirmai[5] and Selam Uqubay[6*]

Berhane *et al. Malar J (2017) 16:105*
DOI 10.1186/s12936-017-1752-9

- **RDTs implemented in 2006: SD Bioline Pf/Pv 05FK80**
- **False negative RDTs reported in 2014-2015**

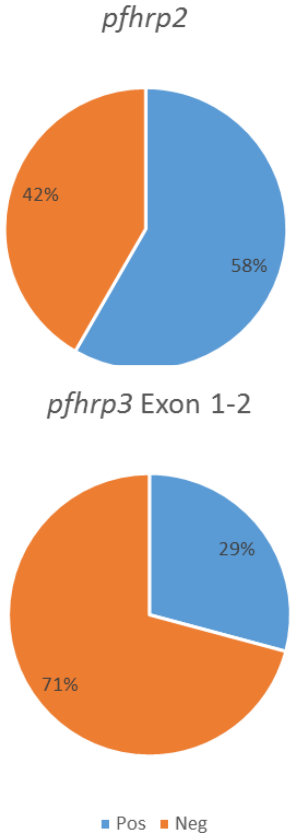
Eritrea MOH team: Araia Berhane; Selam Mihreteab; Salih Mohamed; Filmon Hagos
Australian Army Malaria Institute-QIMR Berghofer: Karen Anderson, Qin Cheng
WHO: Jane Cunningham, Anderson Chinorumba

Ghindae Hospital



n = 26
1,381-89,120 P/µL

Massawa Hospital



n = 24
32 – 25,760 P/µL

***pfhrp2 -/pfhrp3 -* : 42-81%**

Negative Reports

Ghana (unpublished)

- 0 *pfhrp2* deletions found among 165 asymptomatic, PCR-pos/RDT-neg subjects

Kenya (unpublished)

- 0 *pfhrp2* deletions found among 50 asymptomatic, PCR-pos subjects

Unpublished K. Beshir, LSHTM

Asia

pfhrp2/pfhrp3 deletions in India

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Short communication

Genetic deletion of HRP2 and HRP3 in Indian *Plasmodium falciparum* population and false negative malaria rapid diagnostic test

Navin Kumar[a], Veena Pande[b], R.M. Bhatt[c], Naman K. Shah[a], Neelima Mishra[a], Bina Srivastava[a], Neena Valecha[a], Anupkumar R. Anvikar[a,*]

[a] National Institute of Malaria Research (ICMR), Sector 8, Dwarka, New Delhi, India
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[c] National Institute of Malaria Research field unit (ICMR), RLTRI Campus, Raipur, Chhattisgarh, India

ARTICLE INFO

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ABSTRACT

Genetic polymorphisms in diagnostic antigens are important factors responsible for variable performance of rapid diagnostic tests. Additionally, the failure of antigen expression due to gene deletion may also contribute to variable performance. We report Indian *Plasmodium falciparum* field isolates lacking both *Pfhrp2* and *Pfhrp3* genes leading to false negative results of rapid diagnostic tests. The study highlights need to determine the prevalence of *P. falciparum* isolates lacking these genes in larger field populations in India.

Table 1
RDTs and PCR results of *Pfhrp2*, *Pfhrp3* and their flanking gene in laboratory lines and selected *P. falciparum* field isolates.

Lines/isolates	Parasitemia p/μl	RDTs	*Pfmsp1*	*Pfmsp2*	*Pfglurp*	Up stream	*Pfhrp2* exons 1–2	*Pfhrp2* exon 2	Down stream	Up stream	*Pfhrp3* exons 1–2	*Pfhrp3* exon 2	Down stream
3D7	NA	NA	+	+	+	+	+	+	+	+	+	+	+
Dd2	NA	NA	+	+	+	–	–	–	–	+	+	+	+
CB18[a]	47,136	–	+	+	+	–	–	–	–	–	–	–	–
CB21[a]	6952	–	+	+	+	–	–	–	–	–	–	–	–

Note: Only suspected *P. falciparum* isolates out of 48 have shown here, rest of isolates were positive throughout.
(+) Positive result and (–) negative result.
[a] Slide positive but RDTs (Paracheck Pf, Orchid Biomedical System, India and SD Pf, Bio Standard Diagnostics Pvt. Ltd., India) negative samples.

- Conducted in Dec 2010 in Bilaspur district of Chhattisgarh in Central India.
- 48 LM confirmed Pf, with densities: 1800 – 54,448/μL
- 2/48 RDT negative: CB18 and CB21
- CB18 and CB21 failed to amplify pfhrp2, but were successful with amplification of 3 single copy genes

Kumar et al 2013

World Health Organization

pfhrp2/pfhrp3 deletions in India

Prevalence of *pfhrp2* and/or *pfhrp3* Gene Deletion in *Plasmodium falciparum* Population in Eight Highly Endemic States in India

Praveen Kumar Bharti[1], Himanshu Singh Chandel[1], Amreen Ahmad[1], Sri Krishna[1], Venkatachalam Udhayakumar[2], Neeru Singh[1]*

July – Dec 2014
16 sites in eight malaria endemic states in India

Bharti et al 2016

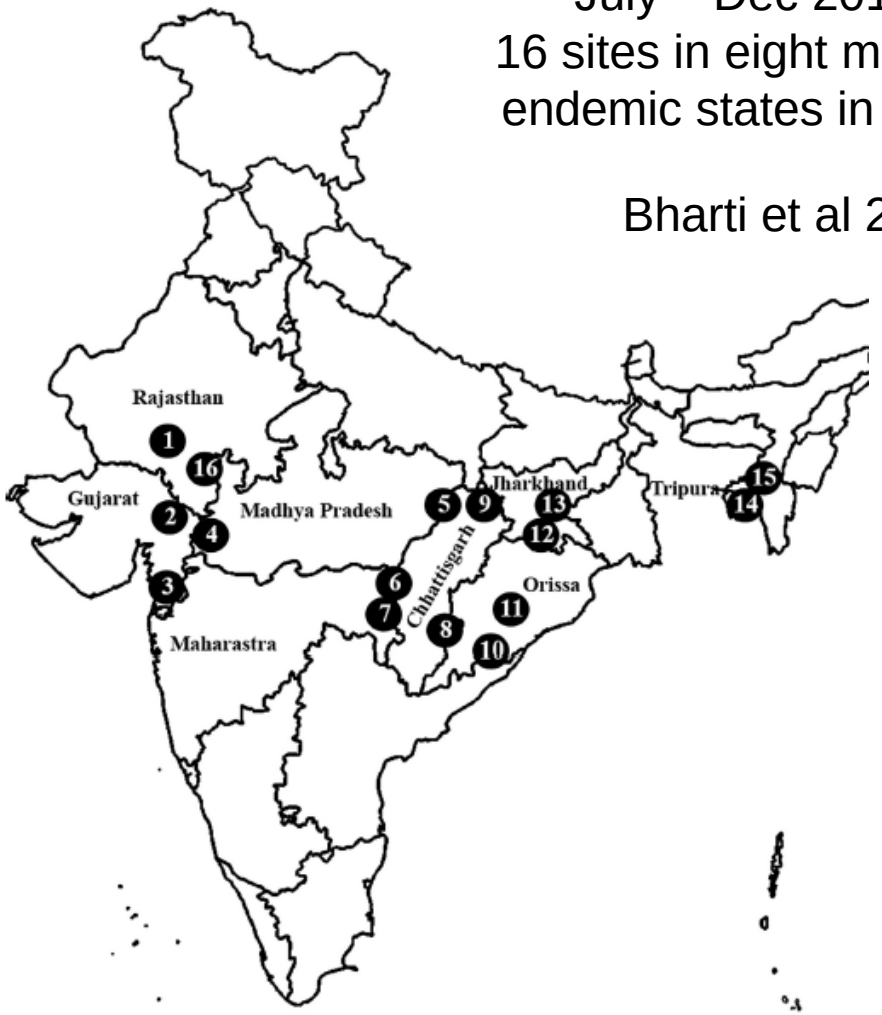

Fig 1. Map showing the study sites from eight malaria endemic states of India. Each state has two study sites.

- HRP2 deletion: 2.4% (36/1521)

Range: 0-25%, 2.4 95% CI: 1.6-3.3

- HRP3 deletion: 1.8% (27/ 1521)
- Both HRP2/3: Range: 0–8% (1.6, 95% CI; 1.0–2.4)

pfhrp2/pfhrp3 deletions: China-Myanmar border

Acta Tropica 152 (2015) 26–31

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Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Genetic diversity of *Plasmodium falciparum* histidine-rich protein 2 in the China–Myanmar border area

Peipei Li[a], Hua Xing[b], Zhenjun Zhao[a], Zhaoqing Yang[c], Yaming Cao[d], Wenli Li[b], Guiyun Yan[e], Jetsumon Sattabongkot[f], Liwang Cui[g,*], Qi Fan[a,*]

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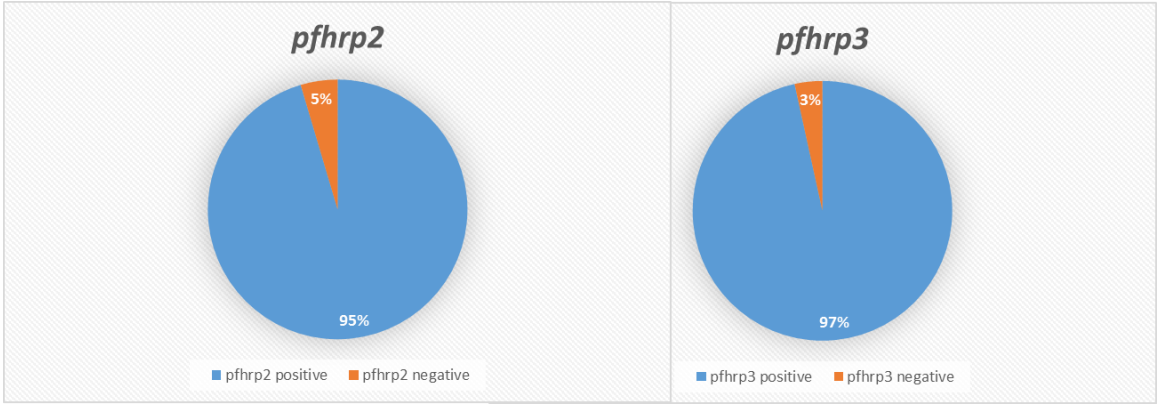
Deletion
Variation

ABSTRACT

Deletion of the *Plasmodium falciparum histidine-rich protein 2* (*pfhrp2*) gene may affect the performance of *PfHRP2*-based rapid diagnostic tests (RDTs). Here we investigated the genetic diversity of the *pfhrp2* gene in clinical parasite isolates collected in recent years from the China–Myanmar border area. Deletion of *pfhrp2* has been identified in 4 out of 97 parasite isolates. Sequencing of the *pfhrp2* exon2 from 67 isolates revealed a high level of genetic diversity in *pfhrp2*, which is reflected in the presence of many repeat types and their variants, as well as variable copy numbers and different arrangements of these repeats in parasite isolates. In addition, we observed *pfhrp3* deletion in three of the four parasites harboring *pfhrp2* deletion, suggesting of double deletions of both genes in these three isolates. Analysis of two cases, which were *P. falciparum*-positive by microscopy and PCR but failed by two *PfHRP2*-based RDTs, did not find *pfhrp2* deletion. Further correlational studies of *pfhrp2* polymorphisms with detection sensitivity are needed to identify factors influencing the performance of RDTs in malaria-endemic areas.

- May 2011 - Dec 2012
- 87 LM confirmed Pf patients from China-Myanmar border, with densities: 40 to105,920/µL
- 4 /87 samples from Myanmar failed to amplify any *pfhrp2* fragments
- 3/4 samples also failed to amplify *pfhrp3*
- All 4 samples amplified 3 single copy genes



Li et al 2015

Bangladesh (submitted for publication)

New

A Case of *Plasmodium falciparum hrp2* gene mutation in Bangladesh

Maisha Khair Nima[1], Thomas Hougard[1, 2], Mohammad Enayet Hossain[1], Mohammad Golam Kibria[1], Abu Naser Mohon[1,3], Rajibur Rahman[1], Rashidul Haque[1], Mohammad Shafiul Alam[1*]

[1]International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka 1212, Bangladesh; [2] Department of Biochemistry, Molecular Biology, and Biophysics, University of Minnesota, Minneapolis, Minnesota, USA; [3]Department of Microbiology & Infectious Disease, Cumming School of Medicine, University of Calgary, Alberta, Canada

24 year old male from Kamalganj Upazilla Health Complex in Sylhet

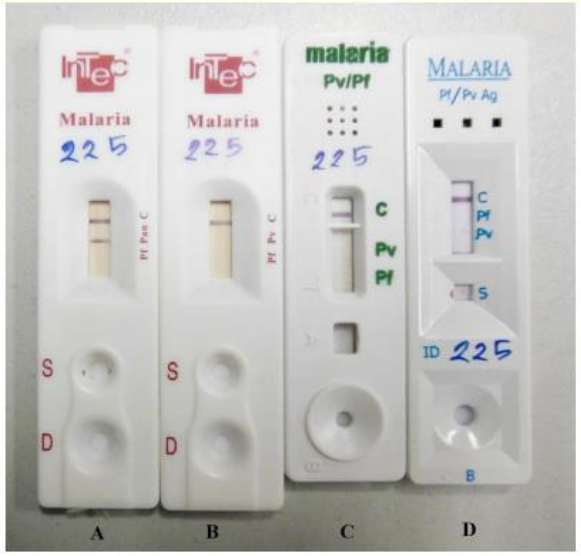


Figure 2: Results in different brands of RDT
A: InTec® Pan/Pf Combo showing PAN(*Plasmodium*) positive *but P. falciparum* negative
B,C,D: InTec® , SD Bioline and Falcivax Pf/Pv Combo showing both *P. falciparum* and *P. vivax* negative

- *P. falciparum* infection confirmed by 18S rRNA PCR
- PCR yielded no visible amplification product for exon 1 of *pfhrp2* gene; exon 2 amplification yielded DNA fragment

No mention of single copy gene amplification…..

World Health Organization

Other

MalGen - Pf3k project analysis for *pfhrp2/3* gene deletions

Countries where we have found HRP2/3 deletions

	Samples	Percent of samples with deletions		
		HRP2	HRP3	Both
Cambodia	856	0.1	4.1	
Ethiopia	24		41.7	
Kenya	122	1.6	0.8	
Laos	121		0.8	
Malawi	262		0.4	
Papua Indonesia	79	3.8	26.6	
Peru	28	28.6	53.6	21.4
Senegal	59		8.5	
Thailand	888		0.4	
Vietnam	216		4.6	

wellcome trust sanger institute

UNIVERSITY OF OXFORD

Global **Malaria** Programme

unpublished: R. Amato, D. Kwiatkowski, *R Pearson*

World Health Organization

‘Report card’

Progress update

1

WHO should promote a harmonized approach to investigating, surveying and reporting *pfhrp2/3* gene deletions through the provision of standard protocols (including sample size calculations) and operating procedures.

A protocol to determine *pfhrp2* gene deletion prevalence among symptomatic individuals with a *Plasmodium falciparum* infection attending public health facilities in being finalized.

********* identify areas with evidence of HRP2 gene deletion prevalence above 5% ********

Key characteristics:

- Province/state will serve as the sampling domain
- Cross-sectional consisting of a systematic random sample of public health facilities selected from a sampling frame of a complete list of all facilities, stratified by facility type and including a measure of facility size, in each province (with transmission).
- All individuals attending the selected facilities with fever and confirmed malaria infection by quality assured pan or pf-pLDH RDTs or microscopy.
- HRP2 (-)/pan or pf-pLDH RDT (or microscopy) (+) patients will be consented for collection of dried blood spot for PCR confirmation of *P. falciparum* infection and *pfhrp2/3* genes
- Brief questionnaire

Sample sizes for determining if the observed HRP2 deletion prevalence is above or below the 5% threshold at the survey domain (province) level

Column 1	Column 2	Column 3
Estimated proportion of HRP2 deletion (outcome 1: total HRP2- & pan-pLDH+ / total LDH+)	Number of individuals needed with P. falciparum infection at province level to conclude 90% CI does not include 5%	Number of individuals with P. falciparum infection per clinic (n=10 clinics per domain)
<1%	150	15
1%	150	15
2%	150	15
3%	350	20
4%	1,550	155
5%	2,280 (assume = 5%)	228
6%	2,280	228
7%	660	66
8%	330	33
9%	210	21
>9%	150	15

$$\text{Observed diagnostic prevalence of HRP2 deletion} = \frac{\text{\# HRP2 discordant results (positive by pan-pLDH, pf-pLDH or microscopy AND negative by HRP2 RDT)}}{\text{\# positive by either diagnostic}}$$

Enroll 150 malaria cases (15 per health facility) If the observed prevalence of HRP2 RDT discordance is at or below 2 or at or above 9%, a total of 150 infected individuals will suffice and enrollment may stop.

Additional survey tools

- Facility tally sheet
- Consent template
- Assent template
- Report forms (patient and laboratory): age, sex, location, travel, antimalarials, RDT, PCR, including electronic data entry tool
- Illustrative study budget
- Tabulation plan for HPP2 prevalence
- Sample size estimator

Reference laboratories

Molecular studies

PCR confirmation of Plasmodium and species ID and hrp2/3 PCR

DNA Sequencing: whole genome, targeted

Institution	Country	Scientist
Australian Army Malaria Institute	Australia	Dr. Q. Cheng
Institut Pasteur	Cambodia	Dr. Didier Menard
National Institute for Research in Tribal Health (NIRTH)	India	Dr. Neeru Singh
MRL/LSHTM	UK	Dr. Khalid Beshir/Colin Sutherland
CDC	USA	Dr. Venkatachalam Udhayakumar (Kumar)
University North Carolina	USA	Dr. Steven Meshnick/Jonathan Parr

Immunoassay (optional)

Elisa

Luminex (HRP2, aldolase, pLDH)

Bead-based immunoassay allows sub-picogram detection of histidine-rich protein 2 from *Plasmodium falciparum* and estimates reliability of malaria rapid diagnostic tests

Eric Rogier, Mateusz Plucinski, Naomi Lucchi, Kimberly Mace, Michelle Chang, Jean Frantz Lemoine, Baltazar Candrinho, James Colborn, Rafael Dimbu, Filomeno Fortes, Venkatachalam Udhayakumar, John Barnwell

Published: February 13, 2017 • http://dx.doi.org/10.1371/journal.pone.0172139

Progress Update 2

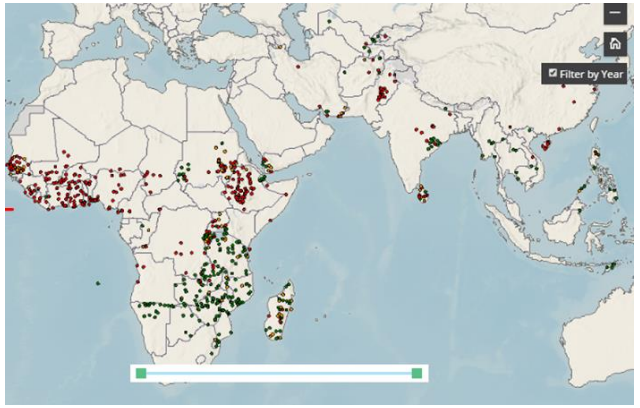
***pfhrp2/3* surveys and surveillance activities should first target countries where deletions or concerns have been identified, and the neighbouring countries.**

- WHO actively supporting the design and planning of surveys for *pfhrp2/3* gene deletions in the states/provinces of Ethiopia and Sudan bordering Eritrea.
- Target implementation during the high-transmission season (September/October 2017).
- Sampling will be powered in order to obtain precise estimates of *pfhrp2/3* gene deletions at the province level
- If< 5% threshold conversion to sentinel site surveillance

Progress Update 3

WHO should integrate information about *pfhrp2/3* gene deletions into the global mapping database

- A review was conducted of all published (and some unpublished) reports of *pfhrp2/3* gene deletions, and data were extracted to inform the online global mapping database under development
- Reports on presence and absence of *pfhrp2/3* gene deletions will be included.
- The review yielded data from 20 countries
- Since the last MPAC meeting, new reports of *pfhrp2/3* gene deletions have emerged from Rwanda, Uganda, Bangladesh and Mozambique



Progress Update 4

The published recommended procedures for investigating and accurately reporting *pfhrp2/3* deletions are comprised of three steps: establishing initial evidence, establishing confirmatory evidence, and establishing prevalence (Cheng Q et al., *Malaria Journal* 2014 13:283).

Revise to recommend

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

WHO information note has been updated to reflect these modifications.

Need to publish in the peer review literature - specifically data quality of recent reports, accurate reporting and thresholds that trigger change.

Progress Update 5

WHO should establish a consortium to provide technical support in investigating suspected false-negative RDTs due to *pfhrp2/3* deletions, to establish appropriate surveillance systems, and to elaborate on factors influencing the emergence and spread of *pfhrp2/3* deletions.

- Network of laboratories has been established to support investigations for pfhrp2/3 gene deletions
  - More resources will be required
- GMP/SUR supporting development of standard survey protocol for determining prevalence of pfhrp2/3 gene deletions – should facilitate incorporation into routine surveillance activity;

Progress Update 6

Tests with both HRP2 and pLDH antibodies on the same test line should be prioritized for assessment by WHO prequalification, including a laboratory evaluation against *pfhrp2/3* single- and double-deleted parasites (culture and clinical samples) to determine whether the tests meet recommended performance criteria.

- Archived materials (7 samples from Peru) and culture adapted *P.falciparum* isolates that do not express HRP2 have been identified
- Prospective collection of wildtype *pfhrp2* deleted parasites is ongoing in Peru (Universidad Peruana de Cayetano Heredia).
- Round 8 of WHO malaria RDT product testing will include a panel of hrp2 deleted parasites (≈30 samples)
  - 9/35 (26%) products in round 8 target pf-pLDH for detection of *P.falciparum*
    - *Manufacturers are responding !!*

- Unfortunately, based on round 7 results one pf-pLDH-combination RDT that previously meet procurement criteria, no longer does and only one new product does meet criteria.

Progress Update 7

Develop a plan of action for surveillance and response that can be supported by partners and implemented in countries.

Action plan should be rooted in an understanding of the extent and spread of deletions and clinical impact ?

- Contents outlined
  - State of knowledge and research gaps
  - Surveillance plan
  - Managing the response & assessing the economic costs
    - Case detection and case management strategies at trigger points (dual testing, etc.)
    - Risk communication with countries/national programmes;
    - Engagement with the diagnostics industry;
    - Procurers (cost constraints, complexity of procuring >1 RDT type and full product replacement);
    - Changes required to WHO Product Testing;
    - Interaction with regulatory/qualifying bodies;
    - Resource mobilization
- Consultant identified
- Ad hoc MPAC review June-July 2017

Mass drug administration for malaria

A practical field manual

Malaria Policy Advisory Committee (MPAC) Meeting

22-24 March 2017, World Health Organization, Geneva, Switzerland

Global **Malaria** Programme

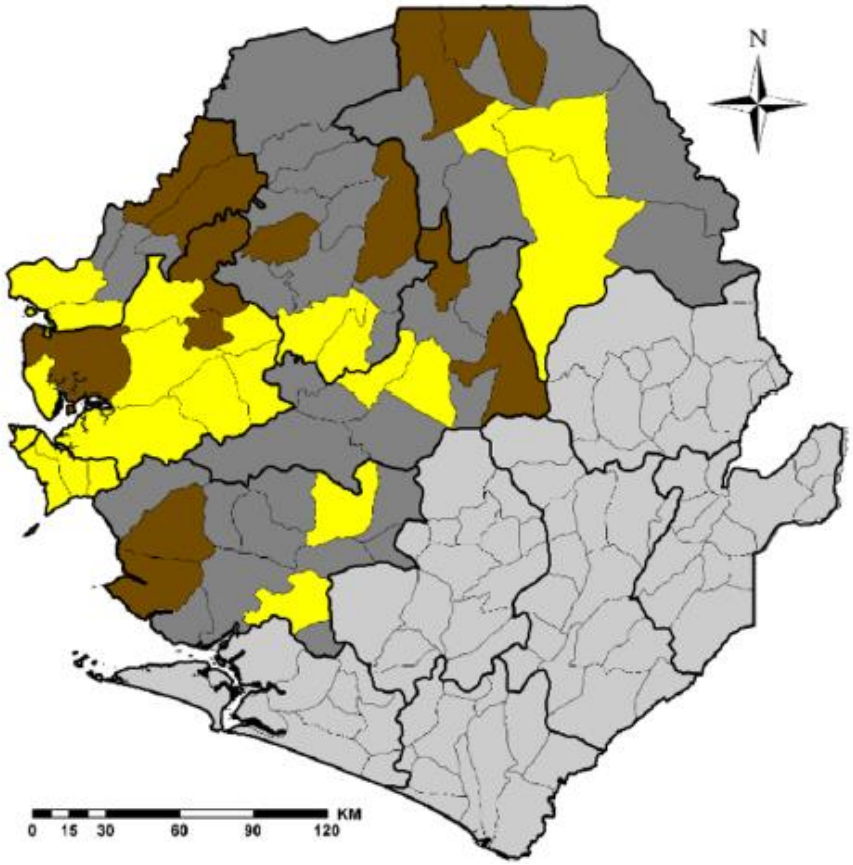
World Health Organization

Background on MDA for malaria

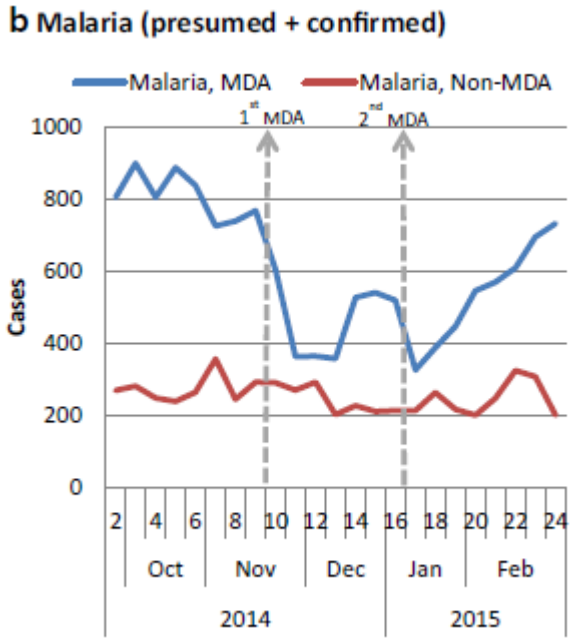
- Over the past decade, mass drug administration (MDA) and other approaches to mass screening and treatment have received increasing interest in the context of malaria elimination and in emergency situations such as the Ebola epidemic in West Africa
- Mass drug administration (MDA) has played a crucial role in the control and elimination of certain prevalent neglected tropical diseases (NTD's) such as lymphatic filariasis, soil transmitted helminthiasis, onchocerciasis, trachoma and schistosomiasis

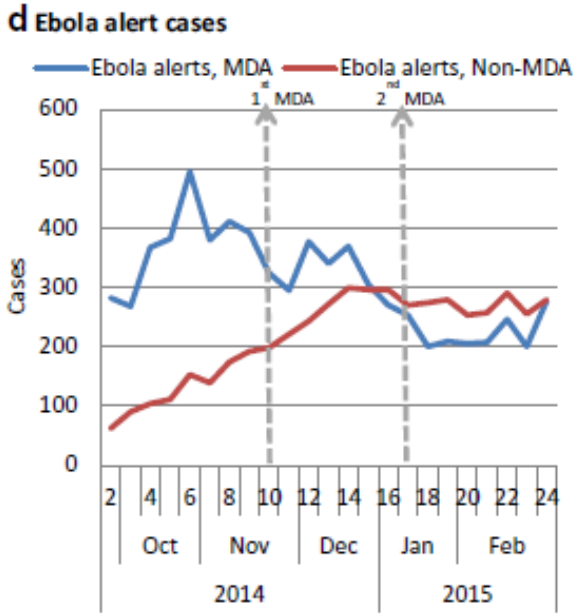
Large-scale MDA for malaria in Sierra Leone

Impact of the MDA for malaria in response to the Ebola outbreak in Sierra Leone. Aregawi *et al. Malar J (2016) 15:480*



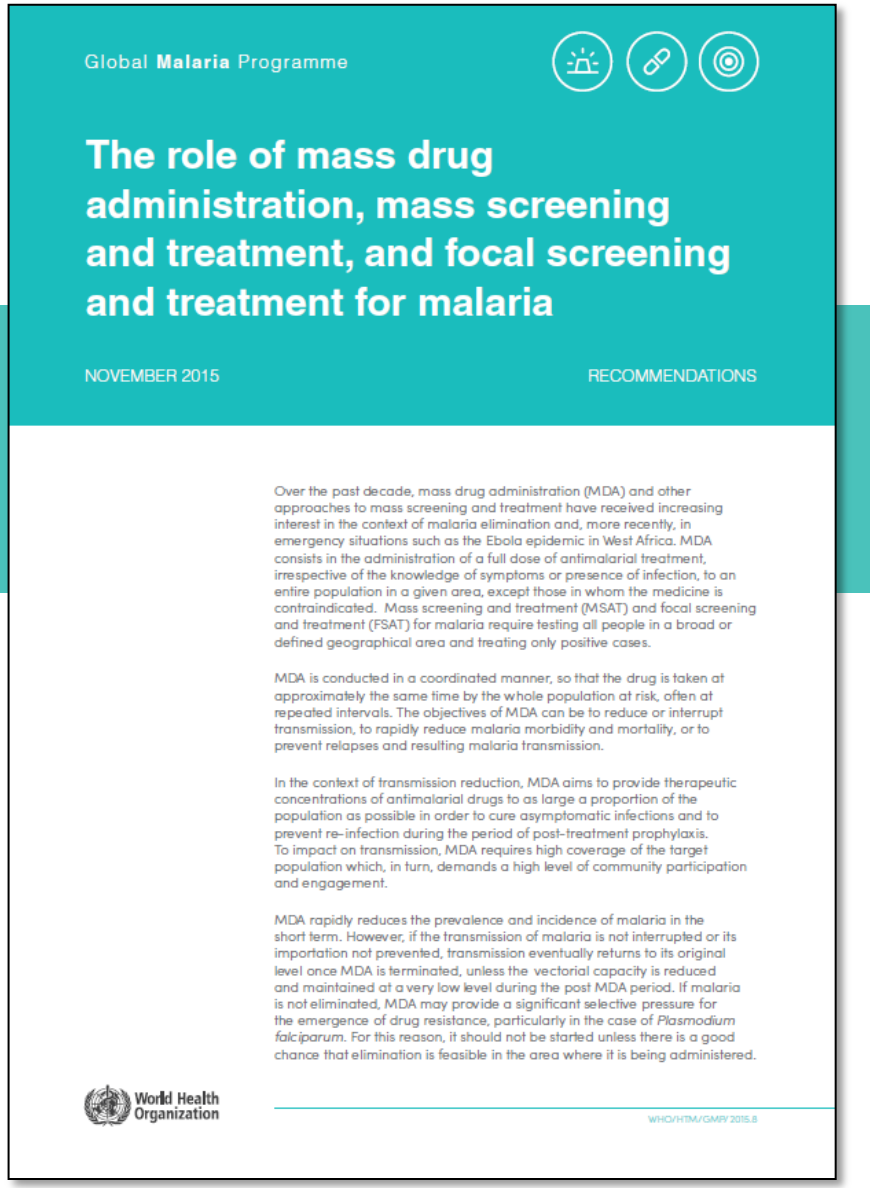
(MDA chiefdoms—*yellow shaded*; and non-MDA chiefdoms—*dark-brown* shaded)





WHO recommendations on MDA

Based on WHO ERG held in April 2015
and MPAC advice in September 2015



Global **Malaria** Programme

The role of mass drug administration, mass screening and treatment, and focal screening and treatment for malaria

NOVEMBER 2015

RECOMMENDATIONS

Over the past decade, mass drug administration (MDA) and other approaches to mass screening and treatment have received increasing interest in the context of malaria elimination and, more recently, in emergency situations such as the Ebola epidemic in West Africa. MDA consists in the administration of a full dose of antimalarial treatment, irrespective of the knowledge of symptoms or presence of infection, to an entire population in a given area, except those in whom the medicine is contraindicated. Mass screening and treatment (MSAT) and focal screening and treatment (FSAT) for malaria require testing all people in a broad or defined geographical area and treating only positive cases.

MDA is conducted in a coordinated manner, so that the drug is taken at approximately the same time by the whole population at risk, often at repeated intervals. The objectives of MDA can be to reduce or interrupt transmission, to rapidly reduce malaria morbidity and mortality, or to prevent relapses and resulting malaria transmission.

In the context of transmission reduction, MDA aims to provide therapeutic concentrations of antimalarial drugs to as large a proportion of the population as possible in order to cure asymptomatic infections and to prevent re-infection during the period of post-treatment prophylaxis. To impact on transmission, MDA requires high coverage of the target population which, in turn, demands a high level of community participation and engagement.

MDA rapidly reduces the prevalence and incidence of malaria in the short term. However, if the transmission of malaria is not interrupted or its importation not prevented, transmission eventually returns to its original level once MDA is terminated, unless the vectorial capacity is reduced and maintained at a very low level during the post MDA period. If malaria is not eliminated, MDA may provide a significant selective pressure for the emergence of drug resistance, particularly in the case of *Plasmodium falciparum*. For this reason, it should not be started unless there is a good chance that elimination is feasible in the area where it is being administered.

World Health Organization

WHO/HTM/GMP/ 2015.8

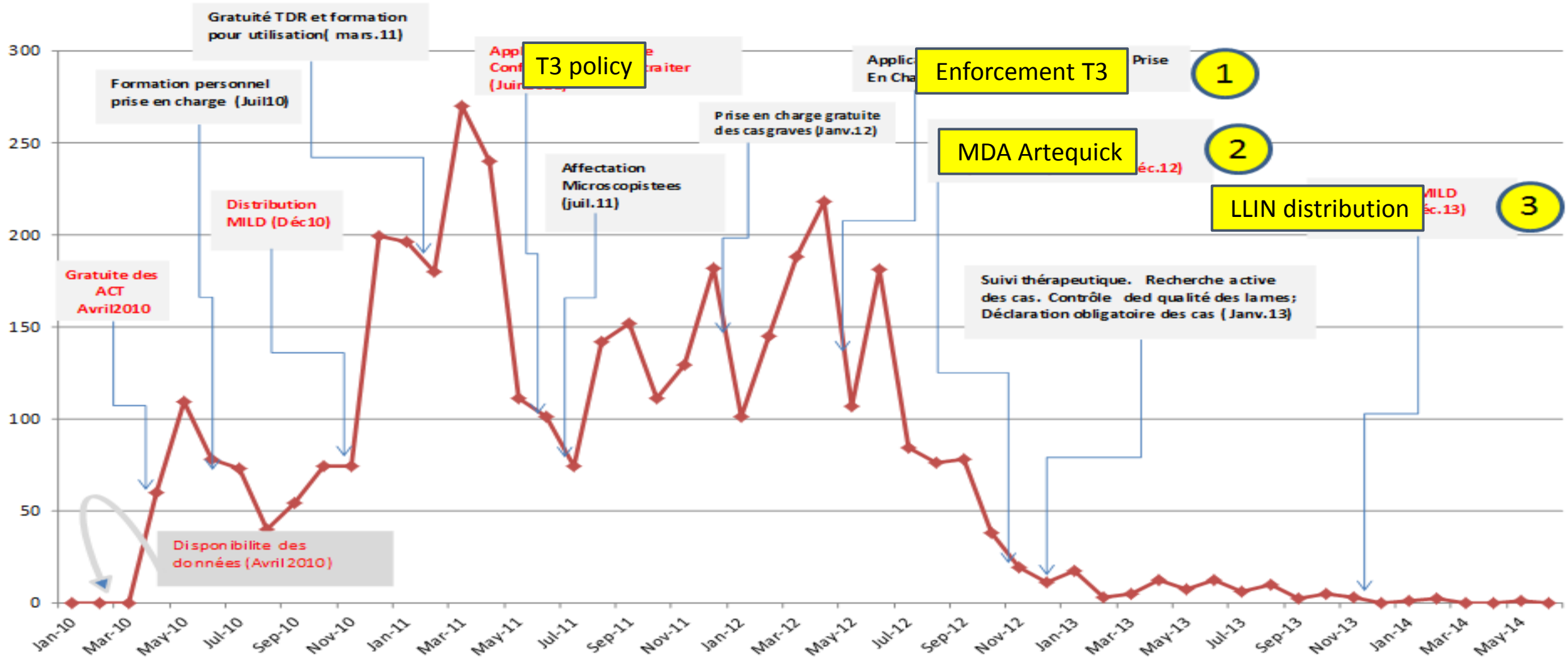
http://www who int/malaria/publications/atoz/role-of-mda-for-malaria pdf?ua=1

WHO recommendations on MDA (I)

Based on a recent evidence review, the WHO Malaria Policy Advisory Committee made the following recommendations on the role of MDA, mass screening and treatment and focal screening and treatment for malaria:

1. Use of MDA for the elimination of *P falciparum* malaria can be considered in areas approaching interruption of transmission where there is good access to treatment, effective implementation of vector control and surveillance, and a minimal risk of re-introduction of infection
2. Given the threat of multidrug resistance and the WHO call for malaria elimination in the Greater Mekong subregion (GMS), MDA may be considered as a component of accelerated malaria elimination efforts in areas of the GMS with good access to treatment, vector control and surveillance

Malaria reported cases in Anjouan, Comores



WHO recommendations on MDA (II)

3. Use of time-limited MDA to rapidly reduce malaria morbidity and mortality may be considered for epidemic control as part of the initial response, along with the urgent introduction of other interventions
4. Use of time-limited MDA to reduce malaria morbidity and mortality may be considered in complex emergencies, during exceptional circumstances when the health system is overwhelmed and unable to serve the affected communities
5. In the absence of sufficient evidence, WHO does not recommend the use of MDA in situations other than for areas approaching elimination, epidemics, and complex emergencies, as specified above (see 1-4)
6. Mass primaquine prophylactic treatment, requiring pre-seasonal MDA with daily administration of primaquine for two weeks without G6PD testing, is not recommended for the interruption of vivax transmission

WHO recommendations on MDA, MSAT and FSAT

7. Mass screening and treatment and focal screening and treatment for malaria are not recommended as interventions to interrupt malaria transmission
8. Medicines used for MDA must be of proven efficacy in the implementation area and preferably have a long half-life WHO recommends that a medicine different from that used for first line treatment be used for MDA Programs should include monitoring of efficacy, safety and the potential emergence of resistance to the antimalarial medicines deployed for MDA
9. WHO supports the need for more research on the optimum methods of implementing MDA programmes, promoting community participation and compliance with treatment, and evaluating their effectiveness Modelling can help guide the optimum method of administering MDA in different epidemiological circumstances and predict its likely impact

MDA: questions from the malaria programs (Dec 2015)

- Definition/Clarity is required
- Checklist for countries
  - Analyze if it works – operational feasibility
  - Determine % *P falciparum* in areas with mixed infections (threshold ?)
  - Population mobility
  - Not for the whole country, but for specific areas/foci/communities
- High coverage expected (total eligible population)
- National treatment guidelines
  - Inclusion of MDA, with indication of medicines to be used for MDA
  - Different chapters for epidemics containment and for malaria elimination (?)
- P vivax?
  - Impact of MDA on P vivax
  - Use of MDA with CQ for P vivax - Primaquine after G6PD testing

Questions on MDA from the programs: planning

- Data availability
- Availability of funds
- Availability of medicines (buffer stock)
- Timing of operations in relation to the malaria season … to be defined
- Use to control malaria epidemics
  - how to define epidemic thresholds
- Target population
  - Eligible population: exclude pregnant women or pregnancy testing?
- Health systems preparedness in detecting & managing ADRs and rumors
- Dosage for children with complex drug regimens – e.g. DHA-piperaquine
- Detailed (minimal essential) guidelines and SOPs

Questions on MDA from the programs: communication

- Information about MDA,
  - Target communities, health workers, policy makers
  - Not as routine intervention, boost for elimination with other interventions
- Information about expected and unexpected side-effects
- Community acceptance
  - 30-50% not willing – don't even start the MDA
- Manage rumors
  - Setting a system for communication in crisis
  - Active detection, management, media briefing and communication dissemination
- Community ownership and engagement

Questions from the programs: monitoring and evaluation

- Monitoring and Evaluation
  - Measure coverage (household members, dispensed drugs, adherence …)
  - Role of molecular methods for parasite detection
  - Surveillance in elimination settings
  - Define indicators by settings – epidemics vs elimination
- When to stop? Emphasize MDA is time-bound
- Need effective pharmacovigilance as part of MDA
- Need for efficacy monitoring, using molecular markers of resistance
- Minimal reporting format
- ……

Process for development of the MDA operational manual

Pre-meeting

- Preparatory phase (before drafting committee meeting)
  - **1st draft** developed by Dr Nanclares based on the MDA experience in Sierra Leone
  - Email review & inputs by 9 members of drafting committee (October – November)
  - **2nd draft** version developed to serve as basis of the drafting commitee

Meeting

- Review by drafting committee in 4 thematic groups (22-23 November)
- Collation of all inputs and development of **3rd draft** version by Rapporteur

Post-meeting

- Email review & inputs by drafting committee (December – January)
- Collation of all inputs and development of **final version** by Rapporteur
- Presentation and discussion at MPAC (March 2017)

Composition/affiliations of participants

Resource persons participating to the Drafting Committee

- Large-scale MDA with AS-AQ for malaria in Sierra Leone — Implementation programs
- MDA with Art-PQP-PQ for malaria in Cambodia and Comoros — Implementation programs
- MDA with DHA-PQP in Magude district, Southern Mozambique — Implementation research
- MDA with DHA-PQP in Southern Province of Zambia — Implementation research
- Research on MDA with DHA-PQP at Thai-Myanmar border — Intervention studies
- Research on MDA with DHA-PQP in Viet Nam — Intervention studies
- Research on MDA with DHA-PQP in Myanmar — Intervention studies
- Community-based pharmacovigilance of ASAQ
- Programmatic experience with MDA for the control of NTD

MDA in the context of transmission reduction for malaria elimination

List of contents of the field guide

FOCUS ON HOW-TO-DO

Key steps in a MDA campaign for malaria

Design phase (macro-planning)	Planning and preparation	Implementation	Monitoring and evaluation	Reporting
• Obtain commitment from policy-makers, and identify agencies to support the ministry of health • Establish a task force or coordinating committee • Conduct a context analysis • Determine target population and geographical areas • Determine antimalarial medicine to be used • Estimate requirements, and order medicine • Determine delivery strategy: o Door to door o Centralized o Mixed • Determine period of intervention • Establish number of rounds • Establish a chronogram • Estimate a budget	• Do micro-planning • Ensure effective logistics - Procurement, storage and distribution - Transport - Accessibility - Distribution sites - Waste management • Human resources - Identify requirements - Training - Salaries and per diem • Community engagement and social mobilization - Define roles and responsibilities - Community assessment - Key messages - Engage mass media - Address rumours - Engage community	• Stock management • Distribution of antimalarial medicine • Supervision • Data collection • Coordination	• Real-time monitoring • Estimation of coverage • Post-campaign survey • Monitoring of consumption • Pharmacovigilance • Monitoring of drug resistance • Evaluation of impact	• Debrief and review intervention • Write a final report

World Health Organization

Executive summary

Planning and preparation phase

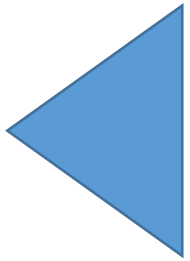
This phase involves planning the operational aspects of the framework defined at national level:

- Conduct **micro-planning at province or district level** according to the strategies defined by the national task force to guarantee an effective campaign by ensuring adequate distribution of supplies, training of staff, engagement of the community and proper management of resources. The micro-plan should include:
  - demographic information on the province or district eligible for MDA
  - information on the area (e.g. maps, infrastructure, location of health facilities, hard-to-reach areas)
  - timing of MDA in the district
  - delivery strategies
  - human resources (number required, number available) and training plan
  - logistical information
  - social mobilization and communications plan and
  - pharmacovigilance plan.

Executive summary (cont'd)

Planning and preparation phase (cont'd)

- Ensure effective logistics, taking into consideration:
  - procurement, storage and distribution of antimalarial medication
  - procurement, storage and distribution of other supplies necessary for MDA
  - transport
  - accessibility to the entire target population, including those in hard-to-reach areas
  - identification and preparation of distribution sites and
  - waste management.
- Plan human and financial resources:
  - **number of teams required and composition**
  - **training and**
  - **adequate payment of salaries and per diem.**



door to door / fixed point
urban / rural
MDA cards / registration
schools, camps, companies…

Executive summary (cont'd)

Human resource requirements

- ***Door-to-door strategy*:** The daily output depend on population density. In urban areas, one team can reach max *75–100 people per day* (average of 15–20 households of five people, visits lasting 15–20 min per household). In rural areas, one team can reach max *50–75 people per day* (10–15 households), considering time for transfer and communication to individual households.
- ***Centralized, fixed-site strategy*:** One team can distribute medicines to *400-500 people per day.* As this strategy is likely to miss a higher proportion of the population than door-to door distribution, special activities are required to mobilize and ensure the participation of the population..
- The daily output depends on whether MDA cards are issued or a registration book is completed, which is more time-consuming.
- Specific teams might be considered for distribution in schools, prisons, military camps and orphanages and perhaps for the main local companies, such as factories, mines and plantations.

Executive summary (cont'd)

Implementation phase

The implementation phase involves the actual distribution of antimalarial treatment and includes:

- stock management: preparation of distribution kits with all the necessary materials ahead of time at the distribution point or peripheral health facility at which supplies are prepositioned
- distribution of the antimalarial medicine itself, either door to door or at a centralized, fixed site
- **supervision, an essential component** to ensure the quality of the campaign: at peripheral, district, regional and national levels
- data collection: collection and reporting of information on the number of people who receive treatment at community level, adverse drug reactions (ADRs) and analysis and compilation of data at higher levels through a well-established pathway of flow of information and
- coordination of all actors to monitor activities, detect any difficulties or constraints, address them and react to unforeseen events.

Executive summary (cont'd)

Monitoring and evaluation

- intra-campaign monitoring system: a high-quality system for monitoring the campaign allows identification of constraints that require immediate action - can be done by monitors identified within the team or by independent monitors
- estimate of distribution coverage: the proportion of the target population that has been reached by distribution
- **post-MDA survey: recommended,** if feasible, after each round or at least at the end of the entire campaign to obtain more reliable information on coverage and to evaluate adherence to treatment, determine reasons for non-participation or non-adherence and evaluate the presentation of ADRs
- monitoring consumption: daily monitoring of the number of treatments distributed and the number taken

Executive summary (cont'd)

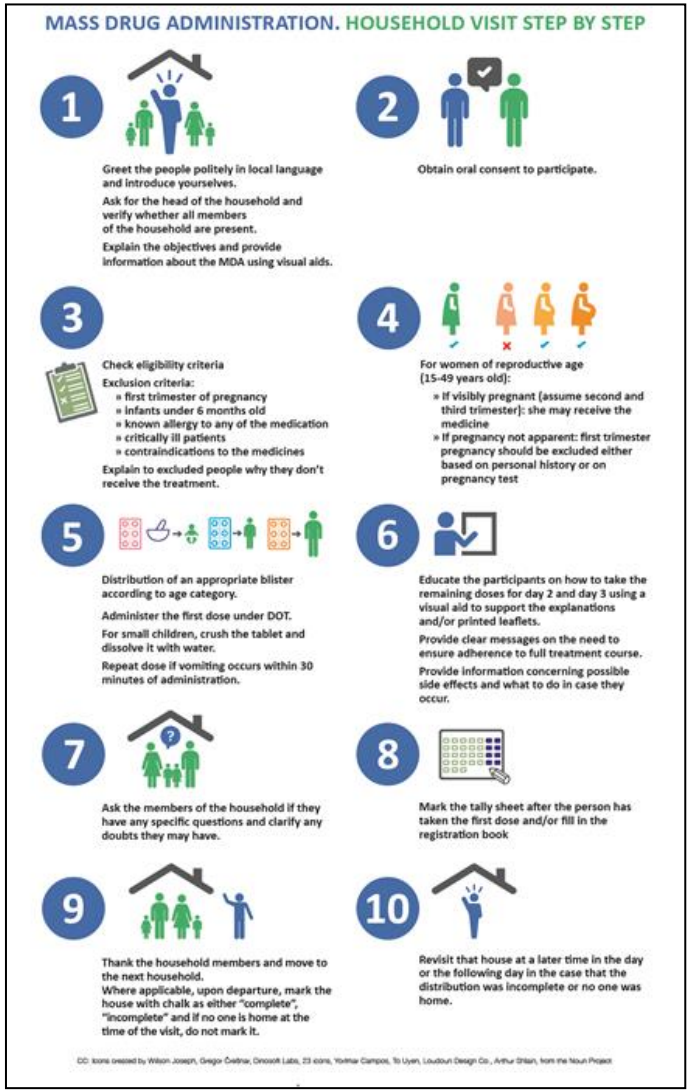
Monitoring and evaluation (cont'd)

- **pharmacovigilance: a vital component of an MDA**, which should be planned to ensure training, detection, reporting, management of follow-up of adverse events and to promote and monitor adherence by both passive and active surveillance. This component is also essential to obtain and maintain good understanding and compliance of the population
- monitoring drug resistance: one of the main concerns with regard to MDA is the emergence and spread of drug resistance although there is no evidence that MDA of artemisinin-based combined therapy (ACT) at therapeutic doses is related to the emergence of resistance, monitoring of resistance should be an essential component of an MDA campaign
- evaluation of impact: through routine surveillance and parasitological surveys and
- reporting: after each round and at the end of the intervention, of the coverage achieved, challenges and difficulties faced and solutions found, lessons learnt, practices with good results, effective social mobilization activities, useful tools and the costs of the intervention.

List of Annexes

Examples of useful tools included in the Annexes

Household visit guide for drug dispensers (Zambia)



MASS DRUG ADMINISTRATION. HOUSEHOLD VISIT STEP BY STEP

1
Greet the people politely in local language and introduce yourselves.
Ask for the head of the household and verify whether all members of the household are present.
Explain the objectives and provide information about the MDA using visual aids.

2
Obtain oral consent to participate.

3
Check eligibility criteria
Exclusion criteria:
- first trimester of pregnancy
- infants under 6 months old
- known allergy to any of the medication
- critically ill patients
- contraindications to the medicines

Explain to excluded people why they don't receive the treatment.

4
For women of reproductive age (15-49 years old):
- If visibly pregnant (assume second and third trimester): she may receive the medicine
- If pregnancy not apparent: first trimester pregnancy should be excluded either based on personal history or on pregnancy test

5
Distribution of an appropriate blister according to age category.
Administer the first dose under DOT.
For small children, crush the tablet and dissolve it with water.
Repeat dose if vomiting occurs within 30 minutes of administration.

6
Educate the participants on how to take the remaining doses for day 2 and day 3 using a visual aid to support the explanations and/or printed leaflets.
Provide clear messages on the need to ensure adherence to full treatment course.
Provide information concerning possible side effects and what to do in case they occur.

7
Ask the members of the household if they have any specific questions and clarify any doubts they may have.

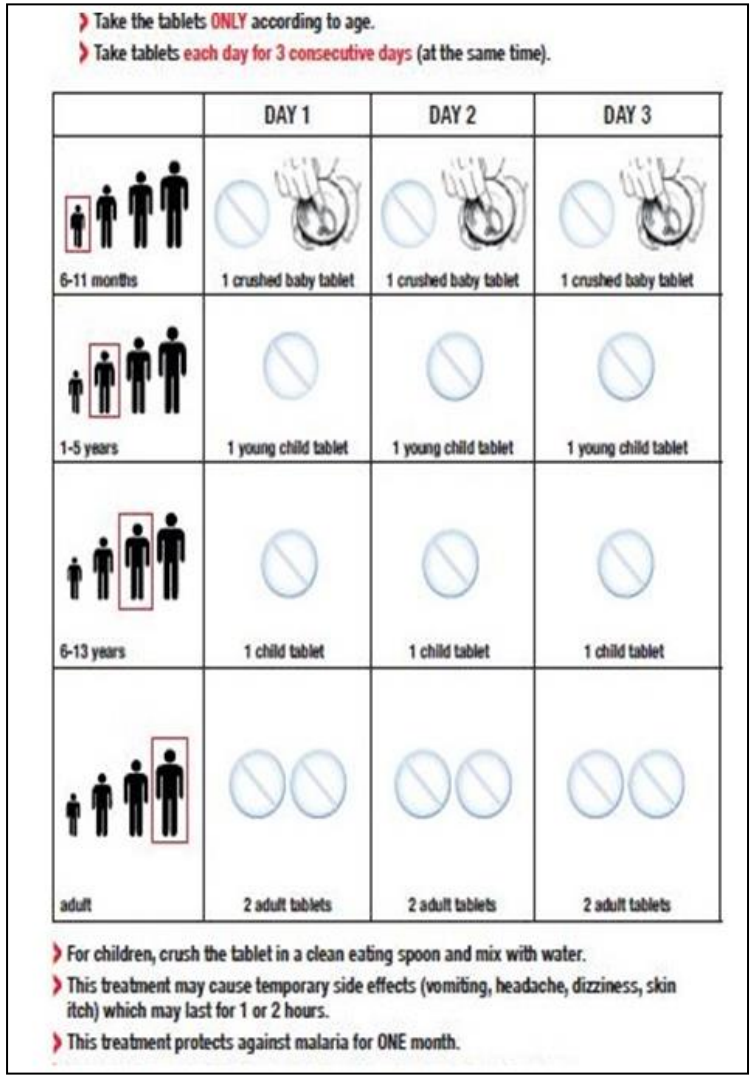
8
Mark the tally sheet after the person has taken the first dose and/or fill in the registration book

9
Thank the household members and move to the next household.
Where applicable, upon departure, mark the house with chalk as either "complete", "incomplete" and if no one is home at the time of the visit, do not mark it.

10
Revisit that house at a later time in the day or the following day in the case that the distribution was incomplete or no one was home.

CC: Icons created by Wilson Joseph, Gregor Cresnar, Oncosoft Labs, 23 icons, Yoelmar Campos, To Uyen, Loudoun Design Co., Arthur Shlain, from the Noun Project

ASAQ dose chart for home visits (Sierra Leone)



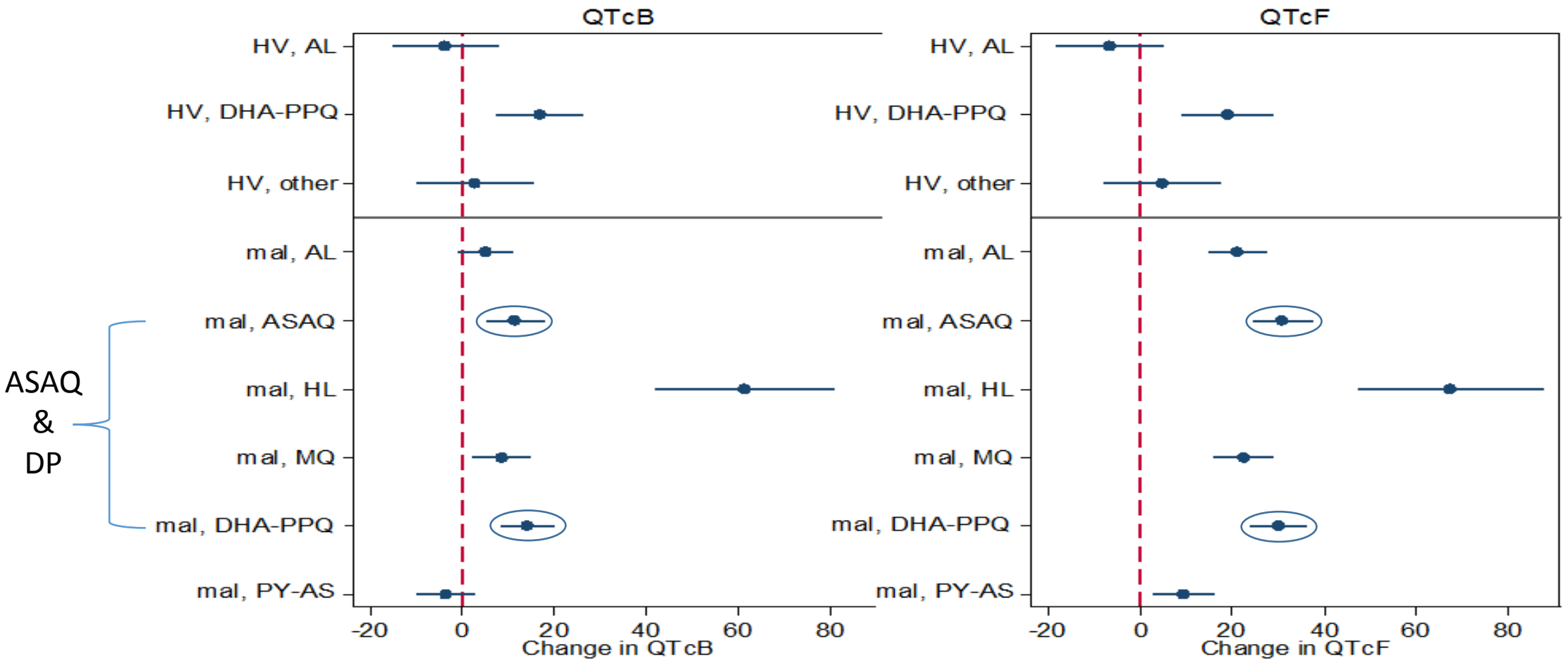
- Take the tablets ONLY according to age.
- Take tablets each day for 3 consecutive days (at the same time).

	DAY 1	DAY 2	DAY 3
6-11 months	1 crushed baby tablet	1 crushed baby tablet	1 crushed baby tablet
1-5 years	1 young child tablet	1 young child tablet	1 young child tablet
6-13 years	1 child tablet	1 child tablet	1 child tablet
adult	2 adult tablets	2 adult tablets	2 adult tablets

- For children, crush the tablet in a clean eating spoon and mix with water.
- This treatment may cause temporary side effects (vomiting, headache, dizziness, skin itch) which may last for 1 or 2 hours.
- This treatment protects against malaria for ONE month.

List of Annexes (cont'd)

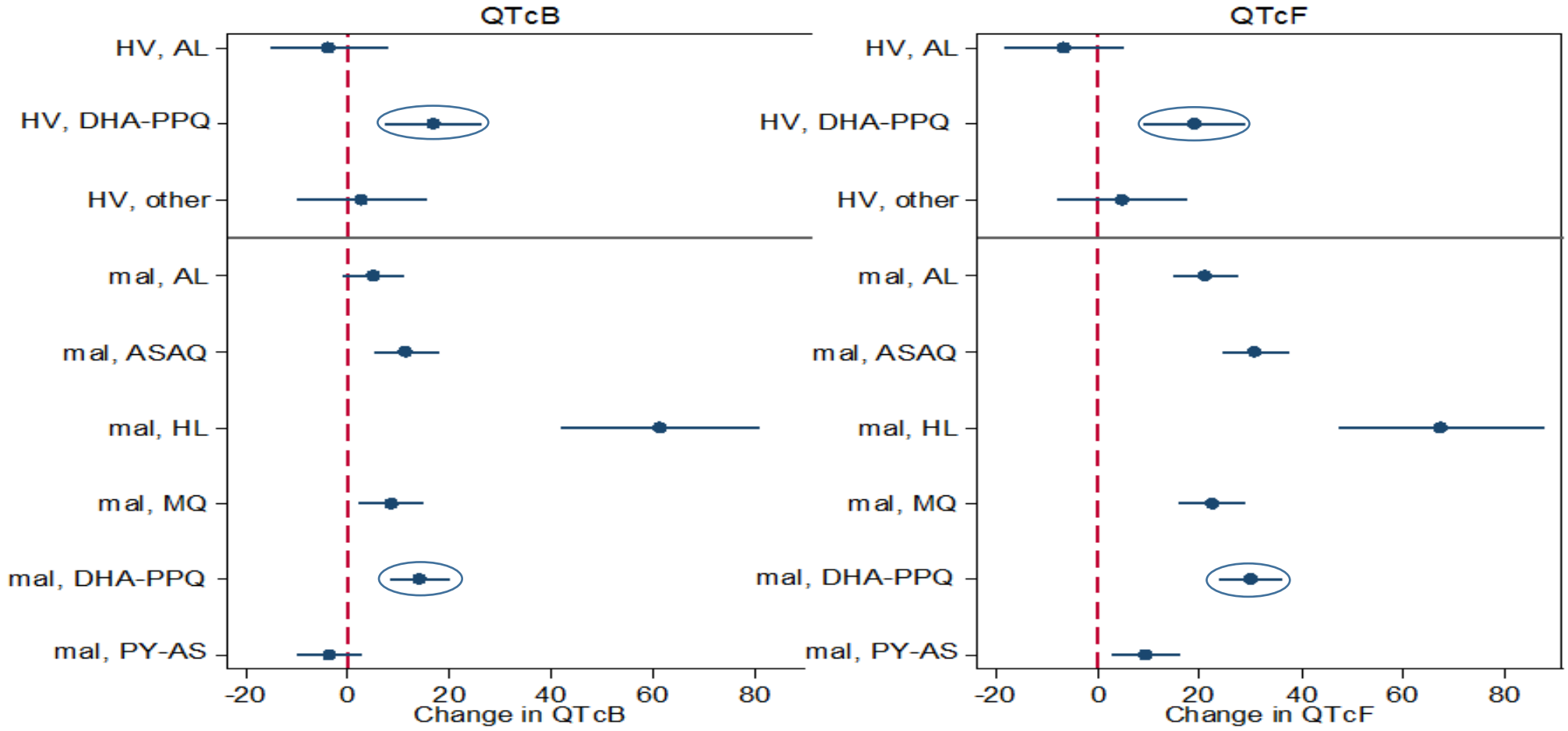
Change in QTc induced by antimalarials



HV = healthy volunteers, mal = patients with uncomplicated malaria.
AL = artemether-lumefantrine, DHA-PPQ = dihydroartemisinin-piperaquine, other = trimethoprim-sulfamethoxazole, ASAQ = artesunate-amodiaquine, HL = halofantrine, MQ = mefloquine / artesunate-mefloquine / artemether-mefloquine, PY-AS = pyronaridine-artesunate.

Change in QTc induced by antimalarials

DP in healthy volunteers & DP in malaria patients



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Relevant findings of the ERG on cardiotoxicity of antimalarials

5. Is the risk of cardiotoxicity after exposure to **piperaquine** containing medicines higher than that of **chloroquine**?

   - **No**. Review of pharmacovigilance, clinical and preclinical data, along with preliminary results of PK/PD modelling, provides no evidence of a significant difference in the risk of cardiotoxicity following exposure to the currently recommended doses of piperaquine, chloroquine or amodiaquine.

6. Is the risk of cardiotoxicity of **piperaquine** containing medicines higher in healthy **volunteers compared to malaria patients**?

   - **No.** Review of pharmacovigilance and clinical data, along with preliminary results from PK/PD modelling, provides no evidence of a difference in the risk of cardiotoxicity of piperaquine-containing medicines in healthy volunteers compared to malaria patients.

Proposed text on cardiotoxicity (to be added in section 3.4.3)

- WHO has recently reviewed the cardiotoxicity of antimalarial medicines. The full report of the Evidence Review Group meeting and the recommendations provided by the Malaria Policy Advisory Committee are available at the following URLs…..
- This review has concluded that the cardiovascular risk associated with the antimalarial drugs piperaquine, amodiaquine, chloroquine is considered very low and these medicines can be used in mass drug administration for malaria.
- As a precautionary principle DHA-PPQ, chloroquine or amodiaquine should not be given for MDA to individuals with a family history of sudden unexplained death consistent with cardiac arrhythmia. Concomitant intake of medicines which prolong the QT interval should be avoided (see http://crediblemeds.org).
- Pharmacovigilance should be strengthened to track and investigate the risk factors associated with sudden unexplained deaths or any other adverse events associated with antimalarial drug use.

Discussion

Overview of WHO policy recommendations for malaria vector control interventions

Malaria Policy Advisory Committee Meeting

Geneva, Switzerland

22 - 24 March 2017

Global **Malaria** Programme

World Health Organization

Presentation overview

1. Rationale for an Information note on existing WHO Policy in the Area of Malaria Vector Control
2. Overview of the Draft GMP Information Note
3. Summary of VCTEG Comments & Advice on Draft Information Note
4. Proposed Revisions & Clarification

Rationale for information note

- WHO process for evaluation of vector control products and associated procedures are being revised
- New process will transfer most product assessment to prequalifications team (PQT), in line with vaccines, drugs and diagnostics
- Existence or absence of a policy for a vector control product submitted to WHO for evaluation will determine pathway and data requirements
- Clarification needed on which products fall under an existing policy and which ones do not

Rationale for information note

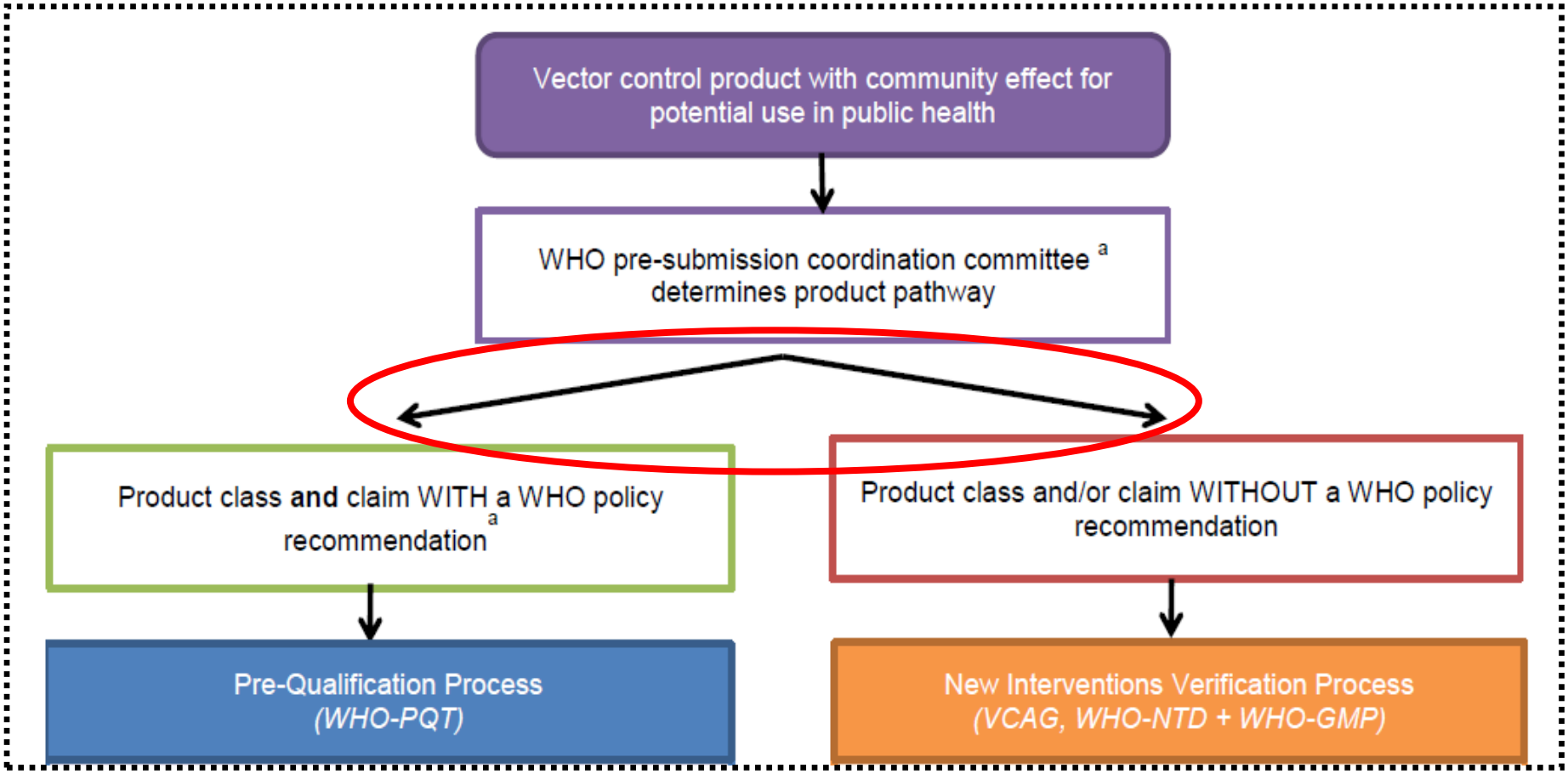


Figure: Top section of draft diagram on WHO process for evaluation of vector control products

Draft information note content

Outline of process

- Data requirements to allow assessment of public health value are determined by Vector Control Advisory Group (VCAG)
- Confirmation of public health value is a pre-requisite for policy development
- VCAG advises WHO on public health value of new tools
- WHO Global Malaria Programme responsible for policy development
- Eligibility for prequalification assessment (and potential listing) requires policy to be in place

Draft information note content: key points

Insecticide-treated nets

- Current WHO policy recommendation pertains to nets with a WHOPES recommendation and that contain only a pyrethroid insecticide
- LLINs containing any other insecticide class or a synergist are not covered under current policy, hence are not eligible for prequalification

Indoor residual spray

- Current WHO policy recommendation pertains to spraying an insecticide formulation with a WHOPES recommendation.
- These formulations contain only one insecticide from one of four classes: pyrethroid, carbamate, organophospate or organochlorine.
- Policy recommendation does not apply to IRS products that contain other insecticide classes or more than one insecticide class. These will require a policy recommendation based on data demonstrating epidemiological impact.

Draft information note content: key points

Supplementary interventions

Larval source management

- Current WHO policy recommendation applies to larvicides with an insecticide formulation that has WHOPES recommendation namely those that contain one of either an organophosphate, an insect growth regulator, a benzoylurea, a spinosyn or a juvenile hormone mimic, or contain one or two bacterial larvicide compounds
- Formulations containing another insecticide class or more than one insecticide class, as well as other larvicidal devices are not covered by current policy

Personal protection measures

- WHO currently recommends three active ingredients1 for personal use
- Other personal protection measures are not currently recommended for broad-scale use in malaria prevention
- Personal protection products currently not eligible for prequalification

[1] DEET (diethyltoluamide), IR 3535 ((3- [N-butyl-N-acetyl], aminopropionic acid ethyl-ester) and KBR3023 (Icaridin or Picaridin).

VCTEG comments (incl. those of observers)

1. More clarity needed on overall process and definition of terms
2. Role of VCTEG in development of policy should be made clear
3. Requirement(s) to prove public health value should be made clear
4. Requirement for a policy recommendation before prequalifications assessment and deployment of new tools was identified as a key issue, as it limits data collection methods and may lengthen time-to-market
5. Randomized controlled trials (RCTs) are expensive and take time. Are they needed for all new tools, or could some be evaluated while being deployed?
6. Current categorization of with/without policy recommendation is based on chemistry, but should be based on product performance. Thinking so far has been around target product profiles (TPP)
7. More clarity is required on how products that are very similar, such as different pyrethroid-only nets will be dealt with (equivalency)
8. More clarity on requirements of products with interim WHOPES recommendation to get a policy recommendation
9. Clarity on what is a supplementary intervention when

VCTEG advice

1. Revise the document to provide more clarity on the process, its different stages, and the requirements for each of these
2. Investigate further how far policy on existing tools could extend to new tools
3. Investigate ways in which the evidence required to determine public health value does not need to come from an RCT and could be generated while a new tool is being deployed

Proposed revision & clarification

1. Overall clarity on process, including definitions of terms, will be covered in a separate document on the overall evaluation process that is under development jointly with NTD and PQT
2. Regarding data requirements for new vector control products we would like to clarify that:
   - To determine whether the first in class of a new product has public health value the standard requirement by VCAG is to assess data from two well conducted RCTs in different and complementary entomological settings, ideally covering two transmission seasons[1]
   - Equivalence will be assesses as outlined in: Determination of Equivalence of Public Health Pesticides and Pesticide Products, WHO 2017

[1] Expert Review Group on trial designs for new vector control tools, will be convened from 24-25 April 2017

Proposed revision & clarification

3. Specific clarification and revision proposed:

LLINs

- As an exception, WHO will accept data from at least one RCT to determine public health value of LLINs with an interim WHOPES recommendation, namely PBO + pyrethroid nets, and pyrethroid + chlorfenapyr nets (Interceptor G2) provided the latter gets an interim WHOPES recommendation during March 2017 meeting. This exception applies only to products submitted to WHOPES in the past and that have been provided with an interim recommendation.
- Products that fall within an established class (e.g. PBO nets once public health value has been established) but vary in their design, will be eligible for prequalification. The entomological data will need to exhibit non-inferiority compared to the "first in class product," thereby demonstrating that the variation in design can be expected to result in similar epidemiological effect.

Proposed revision & clarification

3. Specific clarification and revision proposed (continued):

IRS

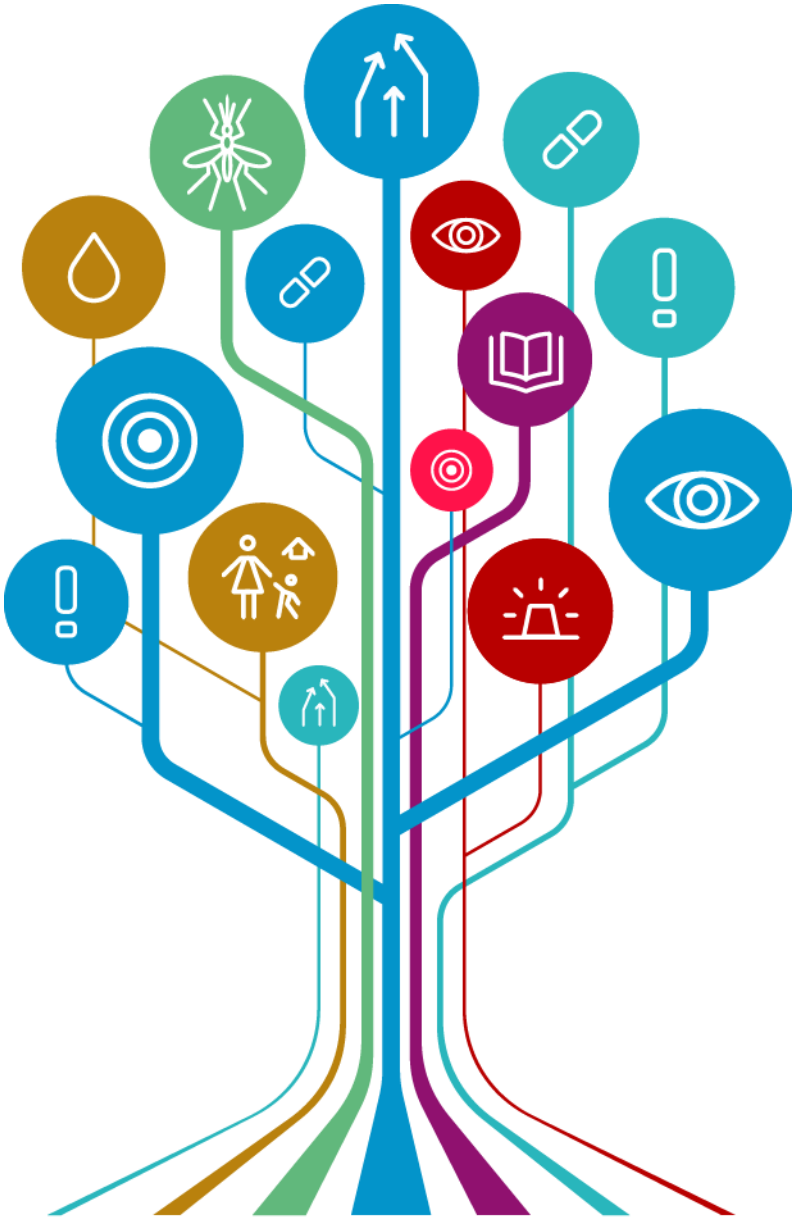
- Option 1: Maintain original wording, i.e. RCT data will be required to assess public health impact of all products that are not a pyrethroid, carbamate, organophospate or organochlorine.
- Option 2:
- Any IRS products that is non-inferior to existing IRS formulations on entomological indicators will be considered as having potential public health value
- Products that perform worse and/or have a different entomological mode of action (e.g. slow acting insecticides or IGRs) compared to existing IRS formulation will be required to provide data from two RCTs to assess their potential public health value

Proposed revision & clarification

3. **Specific clarifications and revisions proposed (continued):**

Larviciding

- Any product that is non-inferior to existing IRS formulations on entomological indicators will be considered as having potential public health value.
- Products with a new entomological mode of action that is distinct from the existing larvicides (OP, PYR, IGRs, Juvenile hormone mimics and Spinosid), will require a stronger evidence-base (to be determined by VCAG).



Global **Malaria** Programme

Malaria Policy Advisory Committee Meeting
22–24 March 2017, Geneva, Switzerland
Background document for Session 8

WHO Evidence Review Group on malaria submicroscopic infections, May 2017

Dr A. Bosman, Dr J. Cunningham
Global Malaria Programme, Prevention Diagnostics and Treatment

Background

In December 2013, WHO convened an Evidence Review Group (ERG) on the role of molecular-based diagnostic techniques for malaria in low transmission areas. The review focused on the different assays available at the time that had a diagnostic performance equivalent or superior to that of microscopy and rapid diagnostic tests (RDTs). On the basis of its review, the WHO recommended the continued use of malaria microscopy and antigen-detecting RDTs for the diagnosis of clinical malaria and routine malaria surveillance (of clinical cases). For epidemiological research and surveys aimed at mapping submicroscopic infections at low transmission intensity and identifying foci in elimination settings, WHO recommends the use of nucleic acid amplification (NAA)-based methods. Furthermore, WHO recommends that tests have a limit of detection of at least 2 parasites/µl. The 2013 ERG did not, however, conduct a detailed assessment of the clinical consequences of submicroscopic infections and their epidemiological contribution to transmission, nor did it provide recommendations on different detection/screening/ surveillance approaches and how to utilize the information emerging from their use.

In recent years, the application of NAA-based diagnostic tools in epidemiological surveys and research has continued to expand; 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. Building on the findings and evidence gaps identified in 2013, WHO's aim is to review new evidence on the role of submicroscopic infections in malaria transmission, as well as the case management and reporting of these infections once they have been detected, in order to provide clear guidance to national malaria control programmes. Where knowledge gaps still exist the group will identify research priorities and propose study designs to evaluate the public health importance of submicroscopic infections and the impact of detecting them using highly sensitive diagnostic tests.

Objectives

1. 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.

WHO/HTM/GMP/MPAC/2017.7

2. 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.
3. 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.
4. 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[1] in March 2014, based on the report of the 2013 ERG meeting[2].
5. 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.

Process

The GMP/PDT unit will collaborate with Dr Teun Bousema, Radboud University Medical Center of The Netherlands, and Professor Chris Drakeley, London School of Tropical Medicine and Hygiene, in inviting relevant research groups to present reviews and original scientific papers addressing the specific objectives listed above. It is not anticipated that research consortia will be established at this time or that funding agencies will be required to sponsor the participation of the presenters and independent reviewers. The same Rapporteur who served for the previous ERG in December 2013 will be contracted to write the meeting report, if available.

The ERG meeting will involve approximately 25 participants and will require 3 days.

A selection of proposed studies to review

Objective 1

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:

1. 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.
2. Tripura R, Peto TJ, Chalk J, Lee SJ, Sirithiranont P, Nguon C, et al. Persistent Plasmodium falciparum and Plasmodium vivax infections in a western Cambodian population: implications for prevention, treatment and elimination strategies. Malar J. 2016;15:181. doi:10.1186/s12936-016-1224-7.

[1] WHO policy recommendation on malaria diagnostics in low transmission settings. Geneva: World Health Organization; 2014 (http://www.who.int/malaria/publications/atoz/diagnotics-low-transmission-settings/en/).
[2] WHO Evidence Review Group on malaria diagnosis in low transmission settings. Geneva: World Health Organization; 2013 (http://www.who.int/malaria/mpac/mpac_mar2014_diagnosis_low_transmission_settings_report.pdf).

3. Imwong M, Stepniewska K, Tripura R, Peto TJ, Lwin KM, Vihokhern B, et al. Numerical distributions of parasite densities during asymptomatic malaria. J Infect Dis. 2016;213(8):1322–9. doi:10.1093/infdis/jiv596.

4. Slater HC, Ross A, Ouédraogo AL, White LJ, Nguon C, Walker PG, et al. Assessing the impact of next-generation rapid diagnostic tests on Plasmodium falciparum malaria elimination strategies. Nature. 2015;528(7580):S94–101. doi:10.1038/nature16040. PMID: 26633771

5. Nsobya SL, Parikh S, Kironde F, Lubega G, Kamya MR, Rosenthal PJ, et al. Molecular evaluation of the natural history of asymptomatic parasitemia in Ugandan children. J Infect Dis. 2004;189(12):2220–6. doi:10.1086/421281.

6. Lin JT, Ubalee R, Lon C, Balasubramanian S, Kuntawunginn W, Rahman R, et al. Microscopic Plasmodium falciparum gametocytemia and infectivity to mosquitoes in Cambodia. J Infect Dis. 2016;213(9):1491–4. doi:10.1093/infdis/jiv599.

7. Pethleart A, Prajakwong S, Suwonkerd W, Corthong B, Webber R, Curtis C. Infectious reservoir of Plasmodium infection in Mae Hong Son Province, north-west Thailand. Malar J. 2004;3:34. doi:10.1186/1475-2875-3-34.

8. Lawniczak MK, Eckhoff PA. A computational lens for sexual-stage transmission, reproduction, fitness and kinetics in Plasmodium falciparum. Malar J. 2016;15(1):487. doi:10.1186/s12936-016-1538-5.

Objective 2

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:

9. Bousema T, Okell L, Felger I, Drakeley C. Asymptomatic malaria infections: detectability, transmissibility and public health relevance. Nat Rev Microbiol. 2014;12(12):833–40. doi:10.1038/nrmicro3364. PMID: 25329408

10. Bousema T, Drakeley C. Determinants of malaria transmission at the population level. In: Wirth D, Alonso P, editors. Malaria biology in the era of eradication. Long Island, NY: Cold Spring Harbor Laboratory Press; 2017.

11. Churcher TS, Bousema T, Walker M, Drakeley C, Schneider P, Ouédraogo AL, et al. Predicting mosquito infection from Plasmodium falciparum gametocyte density and estimating the reservoir of infection. Elife. 2013;2:e00626. doi:10.7554/eLife.00626. *(improved model will be available before the meeting, at least in submitted version)*

12. 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.

13. Johnston GL, Smith DL, Fidock DA. Malaria's missing number: calculating the human component of R0 by a within-host mechanistic model of Plasmodium falciparum infection and transmission. PLoS Comput Biol. 2013;9(4):e1003025. doi:10.1371/journal.pcbi.1003025.

14. Kiattibutr K, Roobsoong W, Sriwichai P, Saeseu T, Rachaphaew N, Suansomjit C, et al. Infectivity of symptomatic and asymptomatic Plasmodium vivax infections to a Southeast Asian vector, Anopheles dirus. Int J Parasitol. 2017;47(2-3):163–70. doi:10.1016/j.ijpara.2016.10.006.

15. Eisele TP, Bennett A, Silumbe K, Finn TP, Chalwe V, Kamuliwo M, et al. Short-term impact of mass drug administration with dihydroartemisinin plus piperaquine on malaria in Southern Province Zambia: a cluster-randomized controlled trial. J Infect Dis. 2016;214(12):1831–9. doi:10.1093/infdis/jiw416.

Objective 3

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.

16. Chen I, Clarke SE, Gosling R, Hamainza B, Killeen G, Magill A, et al. "Asymptomatic" malaria: a chronic and debilitating infection that should be treated. PLoS Med. 2016;13(1):e1001942. doi:10.1371/journal.pmed.1001942.

17. Lindblade KA, Steinhardt L, Samuels A, Kachur SP, Slutsker L. The silent threat: asymptomatic parasitemia and malaria transmission. Expert Rev Anti Infect Ther. 2014;11:6, 623–39. doi:10.1586/eri.13.45.

Proposed Evidence Review Group on malaria submicroscopic infections

Malaria Policy Advisory Committee (MPAC) Meeting

22-24 March 2017, World Health Organization, Geneva, Switzerland

Global **Malaria** Programme

World Health Organization

Presentation Outline

- Background
- WHO recommendations on malaria diagnostics
- Justification for new ERG on submicroscopic malaria infections
- Proposed objectives of ERG
- Discussion

Recommendations on malaria diagnostics

WHO recommendation 1

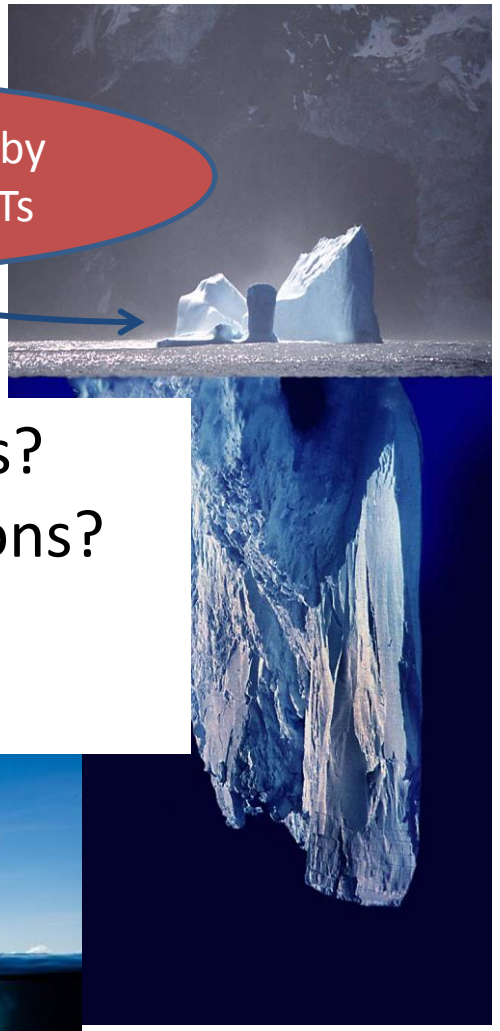
14 March 2014

- 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.

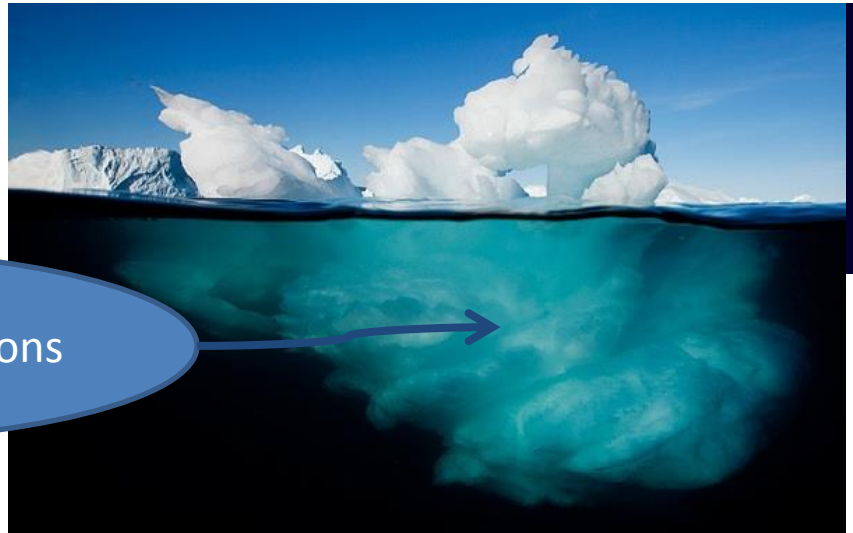
Recommendation 2…6 (related to Nucleic Acid Amplification Techniques)

Detecting sub-microscopic infections

Infections detected by microscopy and RDTs



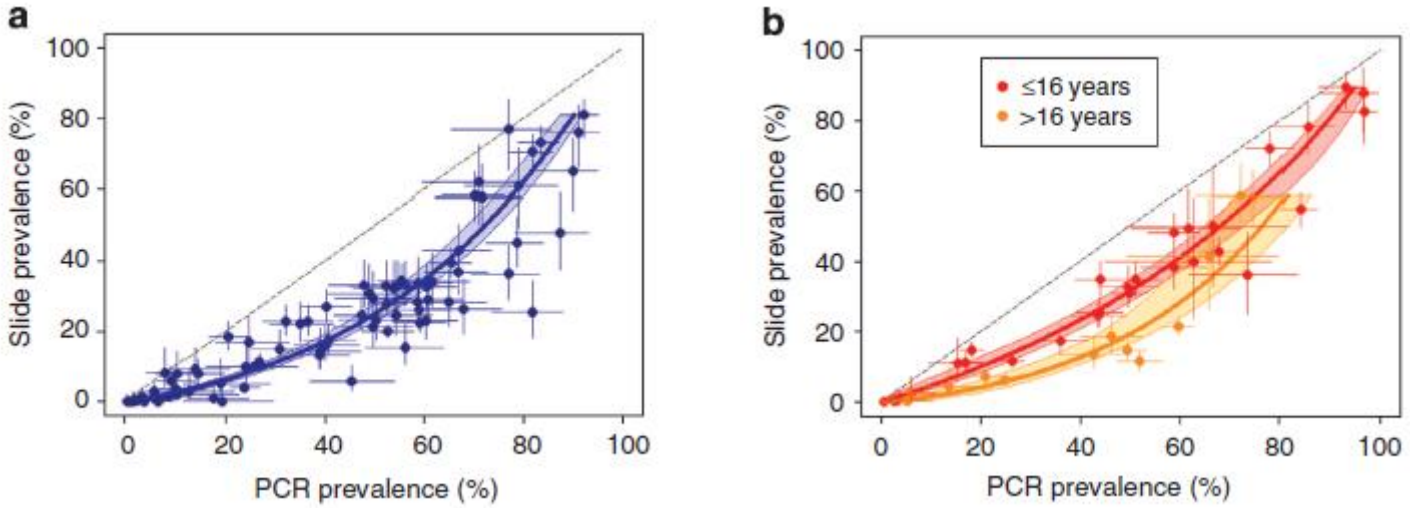
- Who are we missing with microscopy and RDTs?
- What factors influence submicroscopic infections?
- What are their contribution to transmission ?
- When and how to target them?



Submicroscopic infections

Submicroscopic *P. falciparum* infection

Okell et al. Nature Communications (2012) DOI: 10.1038/ncomms2241



- The prevalence of infection measured by microscopy was, on average, 54.1% of that measured by PCR. Submicroscopic parasite carriage more common in adults.

- The gametocyte rate measured by microscopy was, on average, 8.7% of that measured by PCR.

Okell C, Ghani A, Lyons, E et al. JID 2009: 200: 1509-17

WHO current recommendations (cont'd)

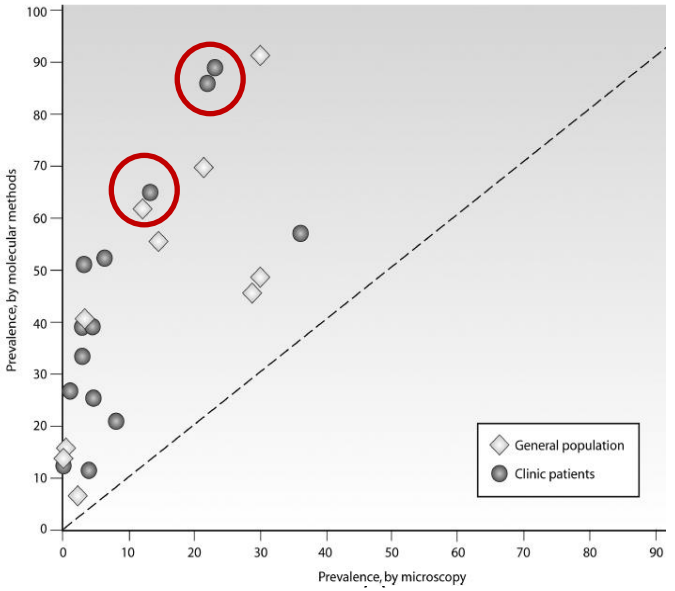
- A number of NAA techniques are available and are more sensitive in detection of malaria compared to RDTs and microscopy. Generally, the use of more sensitive diagnostic tools should be considered only in low transmission settings where there is already widespread implementation of malaria diagnostic testing and treatment and low parasite prevalence rates (e.g. < 10%). Use of 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.
- Submicroscopic *Plasmodium falciparum* and *Plasmodium vivax* infections are common in low as well as in high transmission settings. The use of NAA methods by malaria programs 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.

WHO current recommendations (cont'd)

- 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 mRNA amplification methods in malaria surveys or clinical settings.

Infection = infectious / soon to be infectious

Bousema & Drakeley, Clin Micr Rev 2011



WHO current recommendations (cont'd)

- Common standards for nucleic acid based assays should be developed, including use of the WHO International *P. falciparum* DNA Standard for 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 NAAs are reliable and comparable.
- 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.

NAA methods to detect low parasitaemia

Diagnostic technique	Operational characteristics	Performance[1]	Cost[2]	References
Nested PCR	Uses two sets of primers in successive reactions, therefore increased cost, time and potential for contamination compared to single step PCR.	Limit of detection of at least 6 p/µl for blood spots. Higher sensitivity than single step PCR for four major *Plasmodium* species. Hands-on time 3 hours to result, total time 10 hours.	$1.5-4.0 per sample, $500-5000 for equipment	[24]
Multiplexed PCR	Simultaneous, multiplex PCR to detect the presence of multiple *Plasmodium* species.	Limit of detection 0.2-5 p/µl. 2 hours hands-on time to result, total time 4.5 hours.	$1.5-4.0 per sample (but lower than nested), $500-5000 for equipment	[25-28]
Quantitative PCR	Rapid amplification, simultaneous detection and quantification of target DNA through use of specific fluorophore probes.	Limit of detection 0.02 p/µl for genus level identification, 1.22 p/µl for *P. falciparum* detection. 60 minutes hands-on time to result, total time 2.5 hours.	$4-5 per sample, >$20,000 for equipment	[29-32]
LAMP	Boil and spin extraction can be used, amplification by isothermal method. Result determined by turbidity or fluorescence. Sensitivity can be increased by including mitochondrial targets. Genus level targets, *P. falciparum* and *P. vivax*. Field-appropriate.	Limit of detection 0.2-2 p/µl. Results can be available in 30 minutes with a tube scanner.	$4-5 per sample (commercial), $500-5000 for equipment	[33-37]
QT-NASBA	Assay includes a reverse transcriptase step, less inhibition than PCR. Isothermal method. Can be used for gametocyte quantification. Detects all four *Plasmodium* species, targeting 18S rRNA. Result by fluorescence.	Limit of detection 0.01-0.1 p/µl per 50µl sample. 90 minutes for result (not including extraction time of an additional ~90 minutes)	$5-20 per sample. ? equipment costs	[38-40]

[1] Diagnostic performance influenced by factors including sample preparation, NA extraction efficiency, and amount of blood, amount of template included in reaction, copy number of target sequence, and specific buffers , enzymes etc used.

[2] Cost estimates reported by Erdman LK, Kain KC: **Molecular diagnostic and surveillance tools for global malaria control.** *Travel Med Infect Dis* 2008, **6**:82-99. Cordray MS, Richards-Kortum RR: **Emerging nucleic acid-based tests for point-of-care detection of malaria.** *Am J Trop Med Hyg* 2012, **87**:223-230.

Malaria diagnostics in low transmission

Routine surveillance and passive case detection:

- Based on appropriate case definition of suspected malaria, microscopy and RDTs are sufficient.

Malaria epidemiological surveys :

- Molecular test (or other technology) with analytical sensitivity of ~2 parasites/µl to detect the substantial proportion of low density infections (e.g. classic PCR, qPCR and LAMP or other tests with similar LOD).
- Rapid turnaround is not a priority; internal and external QA is required.

Foci investigations:

- A molecular test (or other technology) with analytical sensitivity of ~ 2 parasites/µl.
- Turn-around time should be <48 hours to allow prompt follow up and treatment of positive individuals; internal and external QA is required.

Malaria diagnostics in low transmission

Mass screening and treatment:

- RDT and microscopy are not sufficiently sensitive
- Molecular test (or other technology) with moderate throughput and analytical sensitivity of ~2 parasites/µl to detect low density infections.
- Results ideally on the same day to maximise follow up and treatment of positive individuals; internal and external QA is required.

Screening of special populations (e.g. at border crossings):

- RDT or microscopy should be used for symptomatic infections only.
- Molecular tests with analytical sensitivity of 2 parasites/µl should be used for detection of infection in asymptomatic individuals.
- Results should be provided on the same day to minimize loss to follow-up.

To be a "significant improvement" over expert microscopy, molecular (and non-molecular) methods needs to be at least one log more sensitive than microscopy i.e. able to detect 2 parasites/µl or fewer.

WHO policy brief on malaria diagnostics

GLOBAL MALARIA PROGRAMME | World Health Organization

Policy brief on malaria diagnostics in low-transmission settings

September 2014

Table of Contents

Background

Light microscopy and rapid diagnostic tests (RDTs) are currently recommended for diagnosis to guide the clinical management of malaria (*1*). Malaria RDTs are used increasingly in many malaria-endemic countries to confirm suspected cases and also for population surveys to monitor changes in malaria transmission.

Nucleic acid amplification (NAA) techniques, which are several orders of magnitude more sensitive than microscopy and RDTs, are increasingly being used in epidemiological studies, investigations of the origin of infections and specific studies such as analysis of parasitaemia in controlled malaria infection trials in humans, drug efficacy trials and drug resistance research. They are also being used to evaluate new strategies and interventions to reduce transmission, i.e. mass drug administration, mass screening and treatment and focal screening and treatment.

At present, WHO considers quality-assured microscopy the gold standard for patient management, even though polymerase chain reaction (PCR) and other NAA assays are more sensitive than microscopy. In view of increasing demand for information on the role of NAA diagnostic tests in malaria, particularly in areas with low transmission, the WHO Global Malaria Programme convened an evidence review group on malaria diagnosis in low-transmission settings, with the following objectives:

(a) to review knowledge about the contribution of *Plasmodium falciparum* and *P. vivax* sub-microscopic parasitaemia to transmission, particularly in areas with low transmission;

(b) to review the diagnostic performance and technical and resource requirements of NAA methods for detecting low-density infections, in order to recommend the most suitable methods for population surveys and active case investigations;

(c) to review the requirements for ensuring the quality for NAA methods and to build capacity in their use in pre-elimination and elimination settings;

(d) to review the current WHO recommendations for malaria diagnostic approaches in low-transmission settings; and

WHO/HTM/GMP/2014.7

The complete policy brief is available on WHO webpage at the following link:

http://www.who.int/entity/malaria/publications/atoz/malaria-diagnosis-low-transmission-settings-sep2014.pdf?ua=1

Background for proposed ERG

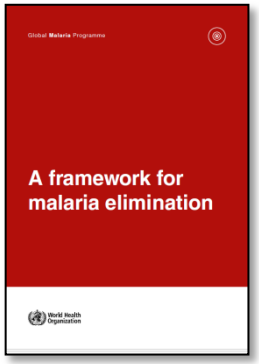
- The 2013 ERG did not conduct a detailed assessment of the natural history of submicroscopic infections and their epidemiological contribution to transmission, nor did it provide recommendations on different detection/screening/surveillance approaches and how to utilize the information emerging from their use.

- In recent years, the application of NAA-based diagnostic tools in epidemiological surveys and research has continued to expand.

- 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.

WHO guidance for malaria elimination

- RDTs and microscopy can be used to detect almost all symptomatic infections and many but not all asymptomatic infections.
- More sensitive diagnostic methods, such as polymerase chain reaction and other molecular techniques, are used to detect asymptomatic infections with very low parasite densities.



- These tests may be useful in surveys for mapping submicroscopic infections, but their value depends on the **epidemiological significance of low-density infections**, which is **not yet sufficiently defined.**
- If local malaria transmission persists despite intensive vector control and universally good case management, the programme may consider undertaking special **studies to evaluate the distribution and frequency** of infections in the asymptomatic population.

Submicroscopic infections in Sri Lanka

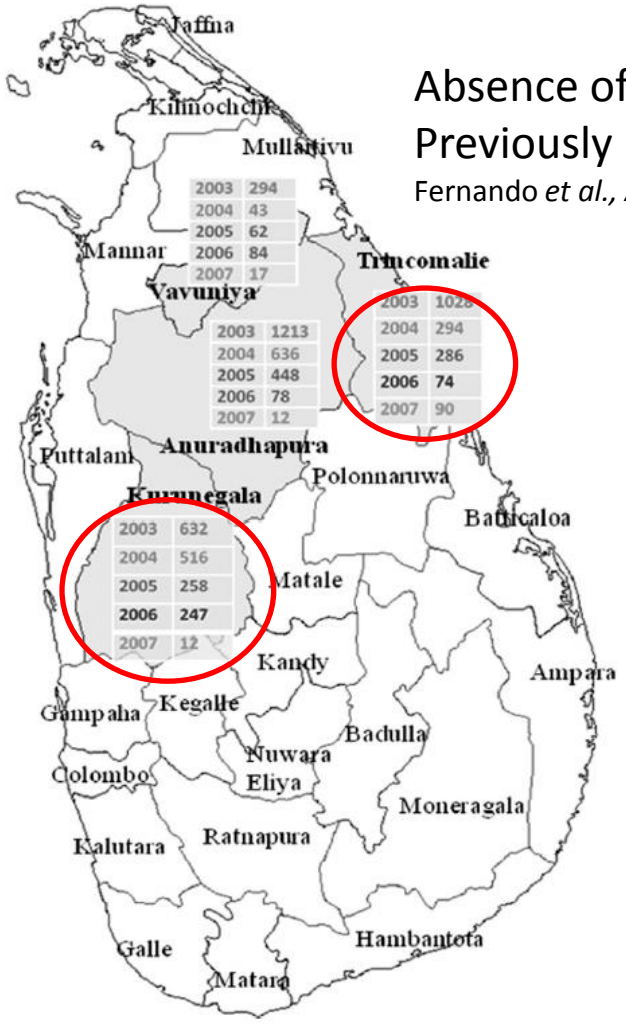
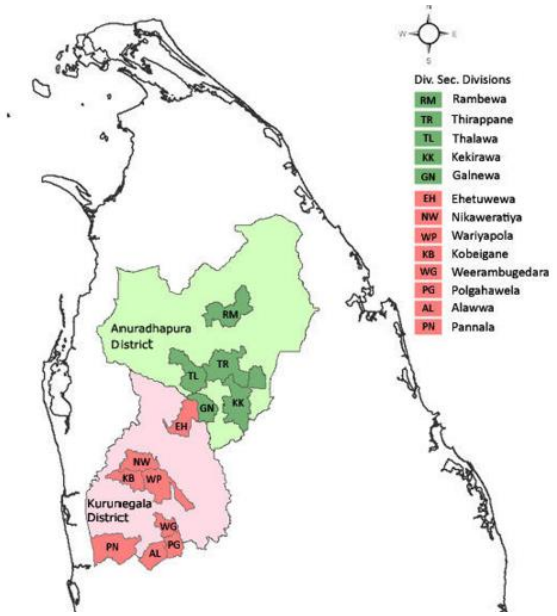


FIGURE 1. Selected districts that reported a higher number of malaria cases during 2003–2007.

Absence of Asymptomatic Malaria Infections in Previously High Endemic Areas of Sri Lanka.

Fernando *et al., Am. J. Trop. Med. Hyg.,* 81(5), 2009, pp. 763–767



Pre-elimination stage of malaria in Sri Lanka: assessing the level of hidden parasites in the population

Rajakaruna *et al. Malaria Journal* 2010, 9:25

Proposed objectives of the ERG

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

2. 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.

3. 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.

Proposed objectives of the ERG

4. 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 .

> • Submicroscopic *Plasmodium falciparum* and *Plasmodium vivax* infections are common in low as well as in high transmission settings. The use of NAA methods by malaria programs 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.

5. 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.

Discussion

Malaria Policy Advisory Committee Meeting
22–24 March 2017, Geneva, Switzerland
Background document for Session 9

World Health Organization

Global call for action to ensure universal access to malaria diagnosis and treatment

March 2017, Geneva, Switzerland

Background

Between 2000 and 2015, great progress was made in extending the coverage of malaria diagnostic testing and treatment (with appropriate antimalarial medicines). Despite this progress, current estimates suggest that large gaps in programme coverage remain, although data are limited. A better understanding as to why these gaps occur, who is affected by these gaps, and what strategies can be used to overcome them will help to ensure that there is universal access to care and that the targets outlined in the *Global technical strategy for malaria, 2016–2030* are met.

Objectives

- To characterize the access to and utilization of malaria diagnostic testing and treatment services at country level, and to identify bottlenecks in service provision (e.g., global supply, access to health facilities, availability of staff and equipment, supply management of commodities, etc.);
- To identify particular population subgroups or risk factors associated with the gaps and the role played by the different delivery channels used to provide services (i.e., public sector, private sector, community-based programmes);
- To review existing datasets and methods used to estimate access to malaria diagnostic testing from routine health management information systems (HMIS) and from health facility and household surveys, and to provide clear methodological recommendations for strengthening the surveillance of malaria testing and treatment;
- To identify effective strategies to increase the access to and utilization of diagnostic testing and treatment services, and to elaborate a global response plan.

Work envisaged

1. **Literature review:** Consult recent peer-reviewed publications and technical reports to prepare a review paper on current access to malaria diagnosis and treatment, including major determinants and gaps. The review will address particular population groups or risk factors associated with gaps in the delivery of services in the public and private sector and through community-based programmes.
2. **Data analysis:** Analyse data on the coverage of malaria diagnostic testing and treatment, particularly from: i) household survey data on diagnostic testing and treatment (especially those surveys where it is possible to link data on history of fever,

WHO/HTM/GMP/MPAC/2017.8

treatment-seeking behaviour and treatment received in relation to malaria test results); ii) health facility survey data on malaria diagnostic testing and treatment; iii) routine HMIS of malaria-endemic countries; and iv) expenditures on and the procurement and distribution of commodities. The paper will review gaps in the evidence and, based on the available data, estimate coverage and examine bottlenecks in the delivery of services.

3. **Economic analysis:** Review the economics of increasing access to diagnostic testing and treatment, by (i) considering the limitations to the supply of and demand for services, and how these can be modified to achieve a more optimal equilibrium; and (ii) examining the costs of alternative strategies.

These three background papers should reflect important regional differences and be prepared in close consultation with WHO Regional Malaria Advisers. They will be shared with all participants 2 weeks prior to the consultation and will serve as the basis for developing a draft global action plan for mobilizing key stakeholders.

4. **Consultation with key stakeholders:** Consult key stakeholders involved in the provision of malaria diagnostic testing and treatment services, including:

   - Representatives of relevant ministry of health (MOH) programmes (essential medicines, malaria, community services, surveillance and central medical stores) from multiple malaria-endemic countries from all WHO Regions;
   - Representatives of technical and funding agencies and NGOs working with MOH programmes to improve access and reporting on malaria diagnostic and treatment services, including in the private sector.

Method of work during the WHO Consultation

a. Analysis of the current situation, determinants and risk groups, based on plenary discussions of the two working papers, with the objective of completing the landscape analysis (Day 1);

b. Working groups on effective strategies for a global response plan to ensure universal access to malaria diagnostic testing and treatment, reflecting different regional/health system contexts (Day 2);

c. Presentation, discussion and consolidation of the main components of the draft WHO global response plan to ensure universal access to malaria diagnostic testing and treatment and to meet the targets set in the *Global technical strategy for malaria, 2016–2030* (Day 3).

End product and WHO endorsement of the draft recommendations

Based on the input received from all participants and the WHO Secretariat, the Rapporteur of the meeting will finalize the draft WHO global plan to ensure universal access to malaria diagnostic testing and treatment. The draft will be submitted to the Malaria Policy Advisory Committee (MPAC) for review and endorsement.

WHO Secretariat of the Technical Consultation

Joint activity between PDT Unit and SEE Team (Drs Bosman and Cibulskis)

Proposed timelines

February–August 2017	Preparation of pre-reads
November 2017	WHO Consultation
February 2018	Finalization of Global Action Plan
March 2018	Review and endorsement by MPAC
25 April 2018	Launch of call for action: World Malaria Day
May–July 2018	Dissemination to WHO Member States and main funding agencies

Global call for action to ensure universal access to malaria diagnosis and treatment

Joint activity of SEE Team and PDT Unit
Andrea Bosman and Richard Cibulskis

Global **Malaria** Programme

World Health Organization

Outline of the presentation

- Background
- Objectives
- Workflow
- Timelines

Problem statement

- There has been great progress in extending the coverage of malaria diagnostic testing and treatment (with appropriate antimalarial medicines) between 2000 and 2015.
- Data are limited but and current evidence suggest that large gaps in programme coverage remain.
- A better understanding of
  - who is affected by these gaps,
  - why these gaps occur, and
  - what strategies can be used to overcome them
- … will help ensure universal access to care and enable the targets outlined in the *Global technical strategy for malaria, 2016-2030* to be attained.

Objectives

1. To characterize the access to and utilization of malaria diagnostic testing and treatment services at country level:
   - to identify particular population subgroups or risk factors associated with the gaps and the role played by the different delivery channels used to provide services (i.e. public sector, private sector, community-based programmes);
   - Identify bottlenecks in service provision (e.g. knowledge, cultural barriers, geographical access, supply chain, financing, regulation, global supply etc);
2. To identify effective strategies (and successful examples) to increase access to, and utilization of, diagnostic testing and treatment services and elaborate a global response plan.
3. To identify data gaps and provide recommendations for strengthening monitoring of malaria testing and treatment.

Planned work – Phase 1

Preparatory work – background paper 1 to cover:

1. Policy and regulation
   - Registration and scheduling of malaria medicines and diagnostics
   - Regulations for providers: private sector and community level
   - Legal and social barriers for populations: migrants, ethnic minorities
   - Financial protection/ exemptions (children under five and pregnant women)
   - Taxes and tariffs for malaria commodities
2. Commodity delivery systems
   - Supply systems – quantification, procurement, distribution (including pull and push systems)
   - Support systems – workforce, information systems (need to consider organization of services e.g. decentralization).

Planned work – Phase 1

Preparatory work – background paper 2 to cover:

1. Funding:
   - Sources of revenue and payment: domestic taxation, international funding, insurance (private, social, community), out of pocket spending
   - Spending on health service delivery and commodity procurement and distribution
   - Financial management

Planned work – Phase 1

Preparatory work – background paper 3 to cover:

1. Need for testing and treatment - number of cases and population groups affected (age/ sex, urban/ rural, socio-economic status etc)
2. Demand for testing and treatment - where people from different groups seek treatment
3. Supply of diagnostic testing and treatment services - including community services and referral systems, provided by different delivery channels
4. → Tanahashi's effective coverage model to identify gaps in coverage and potential actions. Also explore the fraction of potential health gain delivered

Information sources:

- Household survey data
- Health facility survey data
- Routinely reported data
- Supply of commodities
- Health infrastructure
- Qualitative data

Planned work – Phase 2

WHO Technical Consultation on access to malaria diagnostic testing and treatment

Participants:

- Representatives of relevant MOH programs (malaria, community services, surveillance and central medical stores) from multiple malaria endemic countries from all WHO Regions.
- Representatives of technical and funding agencies and NGOs working with MOH programs on improving access and reporting on malaria diagnostic and treatment services, including in the private sector.

Method of work:

- Analysis of current situation based on three working papers, with the objective of completing a landscape analysis (Day 1)
- Working groups on effective strategies to increase access to malaria diagnostic testing and treatment, reflecting different regional/health system context (Day 2)
- Presentation, discussion and consolidation of the main components of a call to action to increase access to malaria diagnostic testing and treatment *(*Day 3)

Planned work – Phase 3

Production of end products:

- WHO global strategy to ensure universal access to malaria diagnosis and treatment
- Technical report on gaps in diagnostic testing and treatment
- Recommendations for strengthening monitoring of malaria testing and treatment

Proposed timelines

- Preparation of pre-reads: March – October 2017
- Review of background papers by MPAC: October 2017
- Technical consultation: November 2017
- Initial draft call to action: February 2018
- Review & endorsement by MPAC (remotely): March 2018
- Launch of technical report and global call for action: World Malaria Day, 25 April 2018
- Launch and dissemination to WHO Member States and Main Funding Agencies: May – July 2018

Discussion