World Health Organization

Friday, 14 March 2014

Time	Session	Purpose	Type
8.30 am 9.00 am 9.30 am 10.00 am 10.30 am	Session 9: TEG Updates Vector Control Advisory Group *(R Velayudhan)* RTS,S vaccine *(P Smith)* Malaria Treatment Guidelines *(N White)* Drug Resistance and Containment *(P Ringwald)* Update on malaria burden estimation and Surveillance, Monitoring and Evaluation TEG *(R Cibulskis)*	For information and input For information and input For information and input For information and input For information and input	open
11.00 am	**coffee**		
11.30 am	Session 10: ERG on malaria diagnosis in low transmission settings Evidence Review Group on malaria diagnostics in low transmission settings – meeting report and recommendations/ Presentation *(K Marsh)*	**For decision (recommendation)**	open
1.00 pm	**lunch**		
2.00 pm	Formulation of MPAC recommendations	MPAC to finalize wording on any recommendations	closed
3.30 pm	**Coffee**		
4.00 pm 5.00 pm	Formulation of MPAC recommendations (cont.) Summary of next steps + agenda for September 2014	MPAC to finalize wording on any recommendations	closed
5.30 pm	**Close of meeting**		

Vector Control Advisory Group (VCAG) – an update

MPAC meeting
WHO HQ, 14 March 2014

Raman Velayudhan and
Abraham Mnzava
World Health Organization
Geneva

Function of VCAG

1. To review and assess the public health value, “proof of principle” (epidemiological impact) of new tools, approaches and technologies; and
2. To make recommendations on their use for vector control within the context of integrated vector management in multi-disease settings.

Attributable benefit: VCAG will provide a clear path for the introduction of forms of vector control

Context

Until now, WHO has not had a comprehensive process for the assessment of new tools, technologies and approaches for vector control

- Policy process historically focused on new products within existing categories of technology (e.g. long-lasting insecticidal nets)
- No defined "entry point" or process for new forms of vector control to receive "proof of principle"

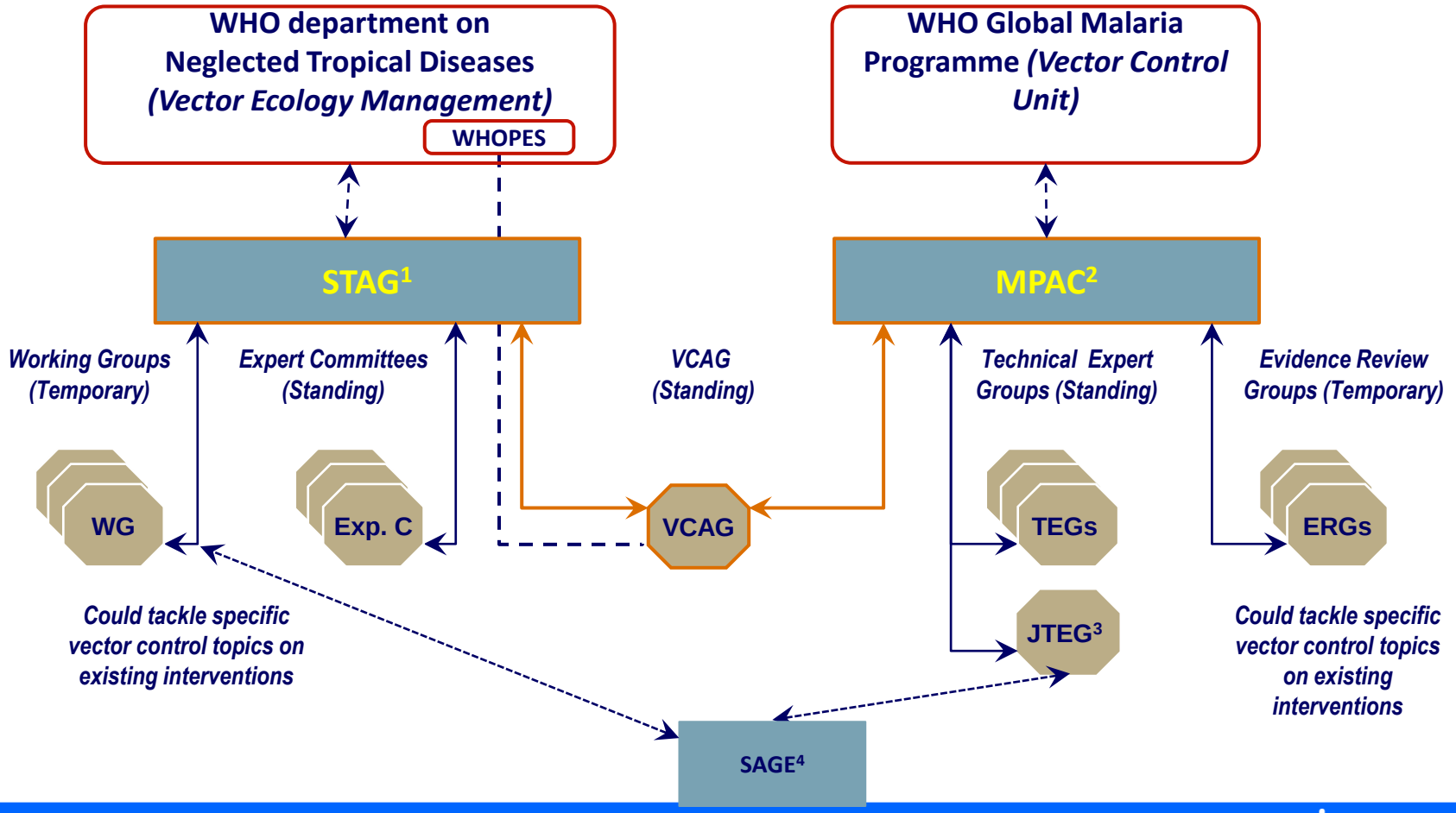
Stakeholders have indicated that the absence of a defined process has, in the past, delayed the adoption and implementation of new forms of vector control

Value-add of VCAG

VCAG will benefit vector-borne disease control by

- Providing a predictable process by which new forms of vector control can be introduced into public health practice (gaining an initial "proof of principle" recommendation)
- Reducing uncertainty for innovators through this clarification
- Accelerating the process of public health implementation of new forms of vector control
- Providing a forum for dialogue and guidance to innovators on evidence requirements early in the process to reduce risks; and
- Providing WHO with evidence-based advice on the epidemiological mode of action and the public health value of new forms of vector control

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



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

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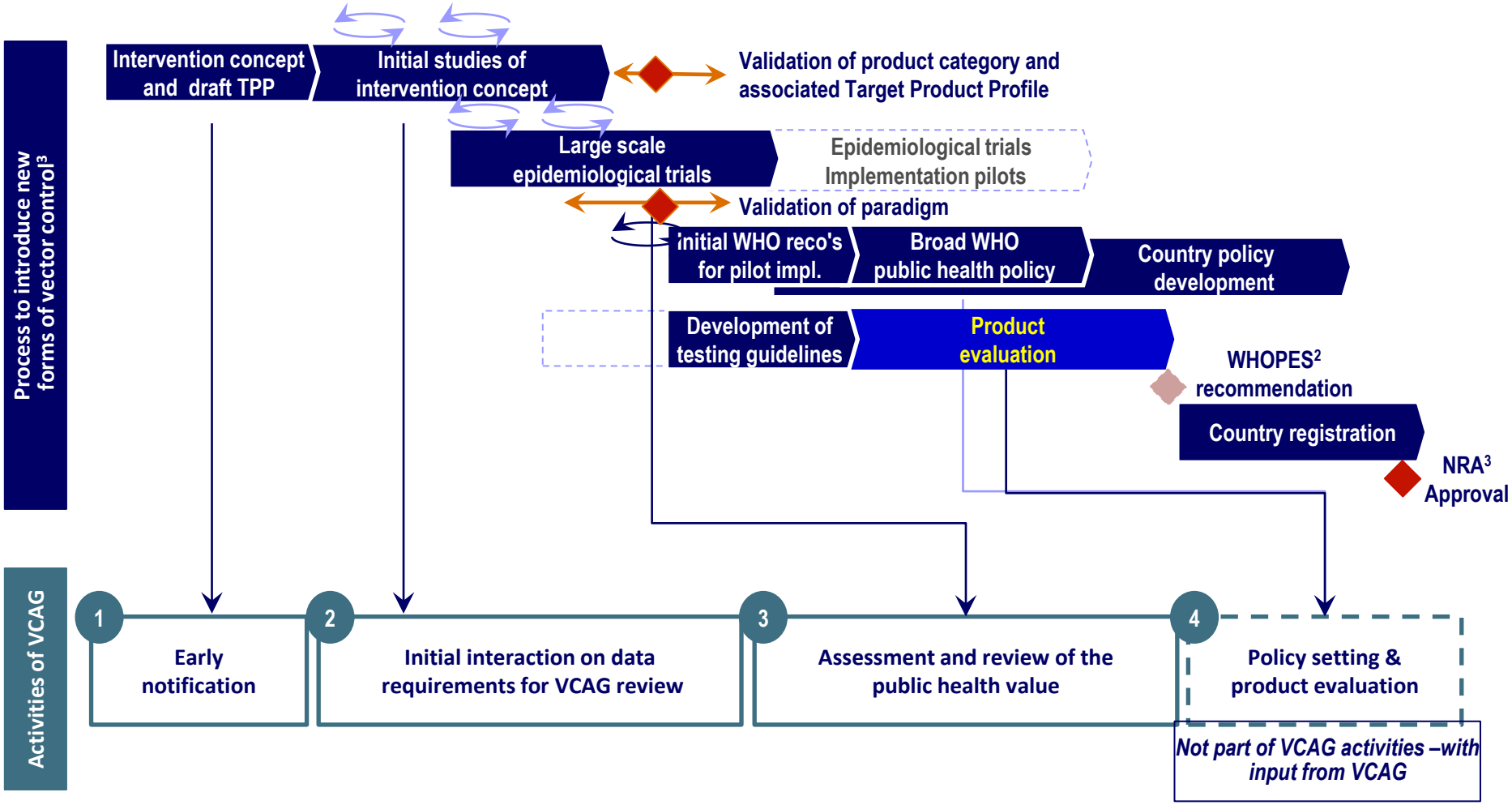
VCAG

- **It is important to note that VCAG will be assessing new classes of technology but will not be involved in considering individual commercial products or the specifications of those products.**

Activities: VCAG could be involved very early in the development of new forms of vector control



1. Made by WHOPES Working Group 2. or equivalent authority; 3. For more details, please see the full mapping document provided as a pre-read to the workshop

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1

Step 1: Notification of intervention to VCAG

Key activities

- The VCAG secretariat accepts and logs notifications of new ideas and intervention concepts being drafted in confidential log, only available to VCAG Members
  - Logs all new projects notified by innovators to create overview of early innovation pipeline, "horizon scanning"
- Based on "horizon scanning", VCAG can comprehend future requirements on process
  - Identify needs for relevant expertise
  - Identify any issues with a new idea early in the process, e.g. develop methods to confirm epidemiological mode of action
- The VCAG secretariat is available to respond to any general inquiries around the VCAG review process
  - E.g. procedures, average timelines etc.

Output

- VCAG Secretariat runs a confidential list of projects early in the pipeline, communicated on a regular basis to VCAG members

Interaction with other groups

- Innovators have the possibility to notify VCAG that a new intervention concept is being drafted / worked on

Step 2: First interaction on data needs for review

Key activities

- If the product developer wishes, the VCAG committee can advise on the type and depth of evidence that will likely be used for its review
  - Provides opportunity for product developer to align with VCAG on overall data requirements before launch of resource-intensive trials
  - Advice provided in individual discussions between innovator and VCAG at the committee meetings
  - May cover need for epidemiological and entomological evidence and mode of action, economic data (to assess intervention feasibility) and data on user acceptability
- To support its deliberations, VCAG may consider the results of tests and studies carried out by product developer
  - E.g. entomological tests and evidence concerning the epidemiological mode of action

Output

- VCAG provides advice to innovators on the type of evidence that will likely be used in the review in step 3

Interaction with other groups

- VCAG receives a request from proposer to get advice on evidence requirements, including a data package with the evidence to date (e.g. results of entomological trials)
- VCAG and IVCC ESAC 3 may also align on what type of evidence will be necessary
  - If IVCC ESAC 3 is involved in the design of trials or other data collection

Source: Interviews

3

Step 3: VCAG review and validation of paradigm

Key activities

- When a dossier has been submitted by the product developer, the VCAG committee consolidates all evidence, and assesses if additional data is needed
  - All sources are consolidated (dossier submitted and other relevant data)
- Based on the evidence, VCAG evaluates the public health value of the new paradigm, by answering:
  - "Is it efficacious, for some defined public health purpose and in some defined circumstances, and will it be useful to and feasible for intended users?"
- VCAG presents its findings to MPAC and STAG, expressing its opinion on the intervention
  - Details the epidemiological mode of action, and the value of the new paradigm in a given setting
  - Product developers informed of VCAG's opinion
- If needed, VCAG may also submit a technical data package to MPAC, STAG and WHOPES for further use in policy and product standard setting

Output

- VCAG advises MPAC, STAG (and WHOPES) on the public health value of the new paradigm, through the expression of opinion
  - Yes / No describing the specific considerations
  - Report and technical data package transmitted
- VCAG may advise product developers on need for additional evidence in some instances

Interaction with other groups

- VCAG may advise the product developer on additional evidence required for a follow-up review
- VCAG presents the outcome of its review in the MPAC and STAG meetings
- VCAG provides input to WHOPES for the consequent definition of testing guidelines

Source: Interviews

4

Step 4: Policy development and WHOPES evaluation

Not part of VCAG activities

Key activities

- Once VCAG submits its report, MPAC and STAG advise WHO on the appropriate guidelines for implementation of the paradigm
  - Establish role of the new intervention for a specific disease, and in relation to other interventions
  - Initial recommendation small scale but incrementally broadened as experience and evidence accumulate
  - Building on VCAG's opinion and technical data package, as well as other available sources
- In parallel, WHOPES convenes experts to develop testing guidelines for the new product category
  - **VCAG's technical package and evidence used as primary input for defining testing guidelines**
- When a specific product within the product category is submitted for evaluation, WHOPES reviews its dossier and commissions any complementary trials necessary for the evaluation
  - **If the product is "first-in-class" and data on the specific product was evaluated by VCAG, WHOPES will build on VCAG's work, taking the evidence from VCAG fully into account to avoid duplication of efforts**

Output

- **GMP and NTD publish guidelines for implementation of the paradigm based on advice from MPAC and STAG, respectively**
- **WHOPES publishes product category testing guidelines, and product recommendations for specific products**

Interaction with other groups

- **MPAC and STAG request additional information from VCAG, if required for the policy development**
- **WHOPES communicates with VCAG around the definition of testing guidelines**
- **WHOPES may advise product developer that additional evidence is required for the product evaluation**

Source: Interviews

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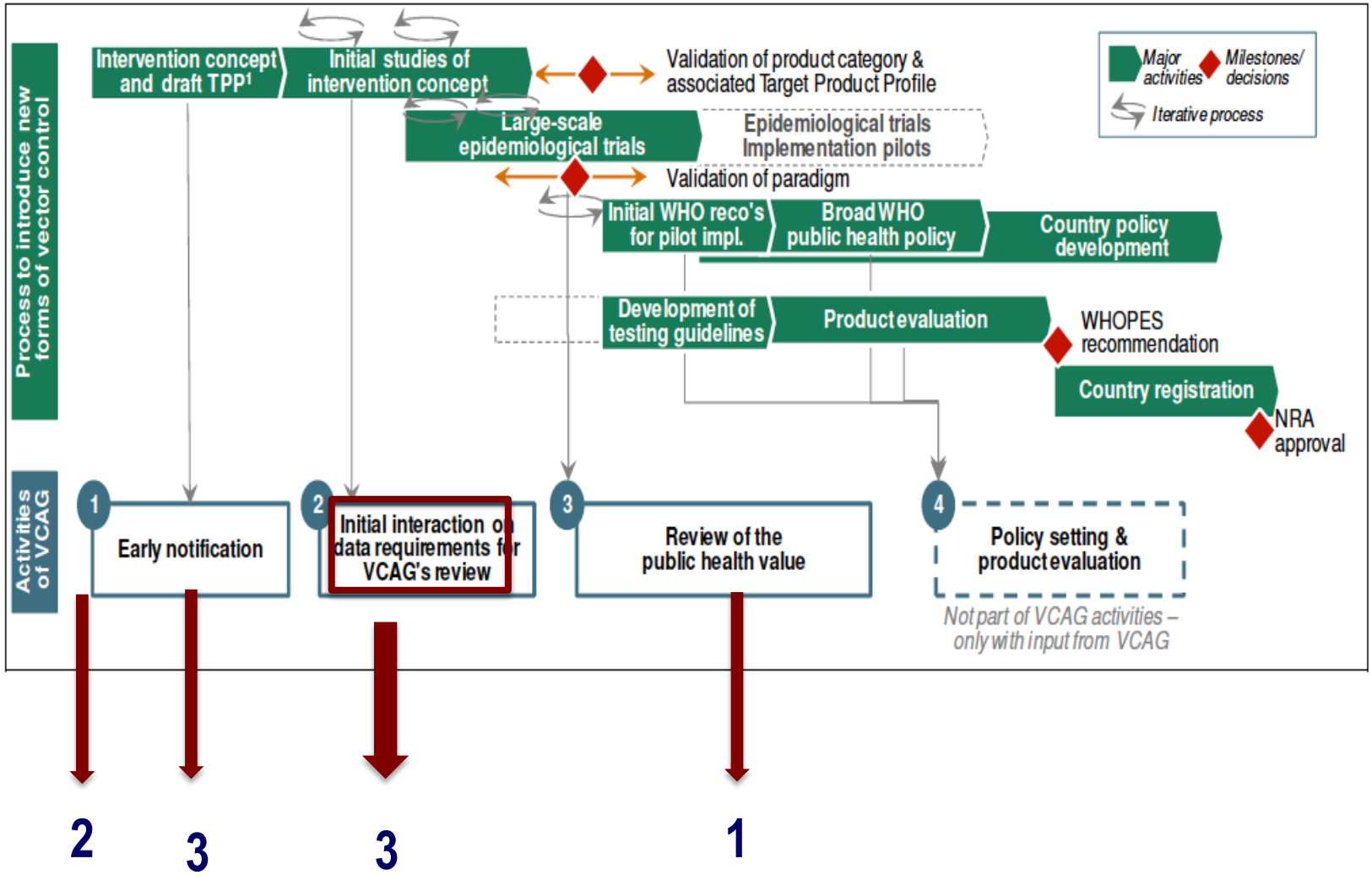
Summary

- Providing a predictable and defined process by which new forms of vector control can be introduced into public health practice
- Reducing uncertainty for innovators through this clarification
- Accelerating the process of public health implementation of new forms of vector control
- Providing a forum for dialogue and guidance to innovators on evidence requirements early in the process to reduce risks.

VCAG submissions

1. Wolbachia	2
2. Spatial repellent	2
3. Attractive Lethal Ovitraps	2
4. PermaNet 3.0	3
5. Smart Patch	1
6. Electrostatic coating	1
7. Ovitraps	1
8. Mosquito free trap	E
9. Non-chemical insecticidal fabrics	E

VCAG engagement



Thank you

WORLD HEALTH DAY APRIL 7TH 2014

"VECTOR BORNE DISEASES"

Articulation: proposed interaction between VCAG and MPAC/STAG in the development of public health policy

Illustrative options to clarify the interactions between VCAG and MPAC/STAG
[Propositions for consideration and modification by MPAC/STAG]

VCAG presents findings to MPAC and/or STAG

Limited evidence

MPAC/STAG may advise on data needs

Option 0: Additional evidence required
- Direct deliberations on data needs by MPAC/STAG and write-up of needs/pilots following VCAG's presentation

Significant evidence

MPAC/STAG may develop policy recommendations

Option 1: Low complexity and/or narrow application
- Direct deliberations and a policy statement write-up by the committee following VCAG's presentation

Option 2: Some complexity or broader application
- Policy statement prepared overnight by "drafting committee", deliberations on next day

Option 3: Complex issue and/or broad application
- MPAC/STAG secretariat asked to prepare draft statement, to be circulated post-meeting to committee members

Option 4: Highly complex issue and broad application
- MPAC/STAG requests an Evidence Review Group, Expert Committee or Working Group to prepare a policy statement to be submitted at next meeting

RTS,S/AS01: Update on JTEG Assessment and Preparations for Policy Recommendations

Peter Smith, Chair JTEG

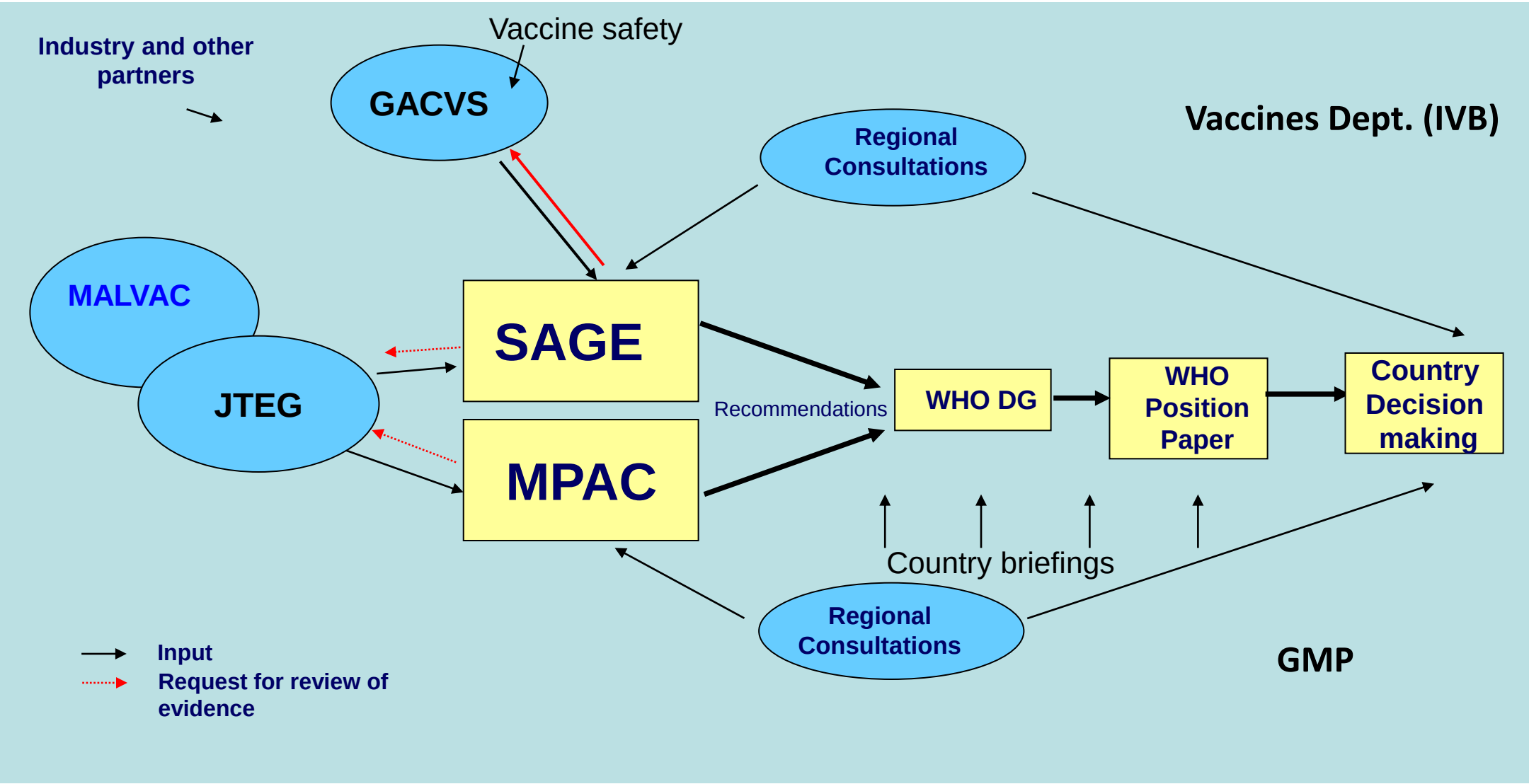
(Vasee Moorthy: WHO staff)

JTEG members

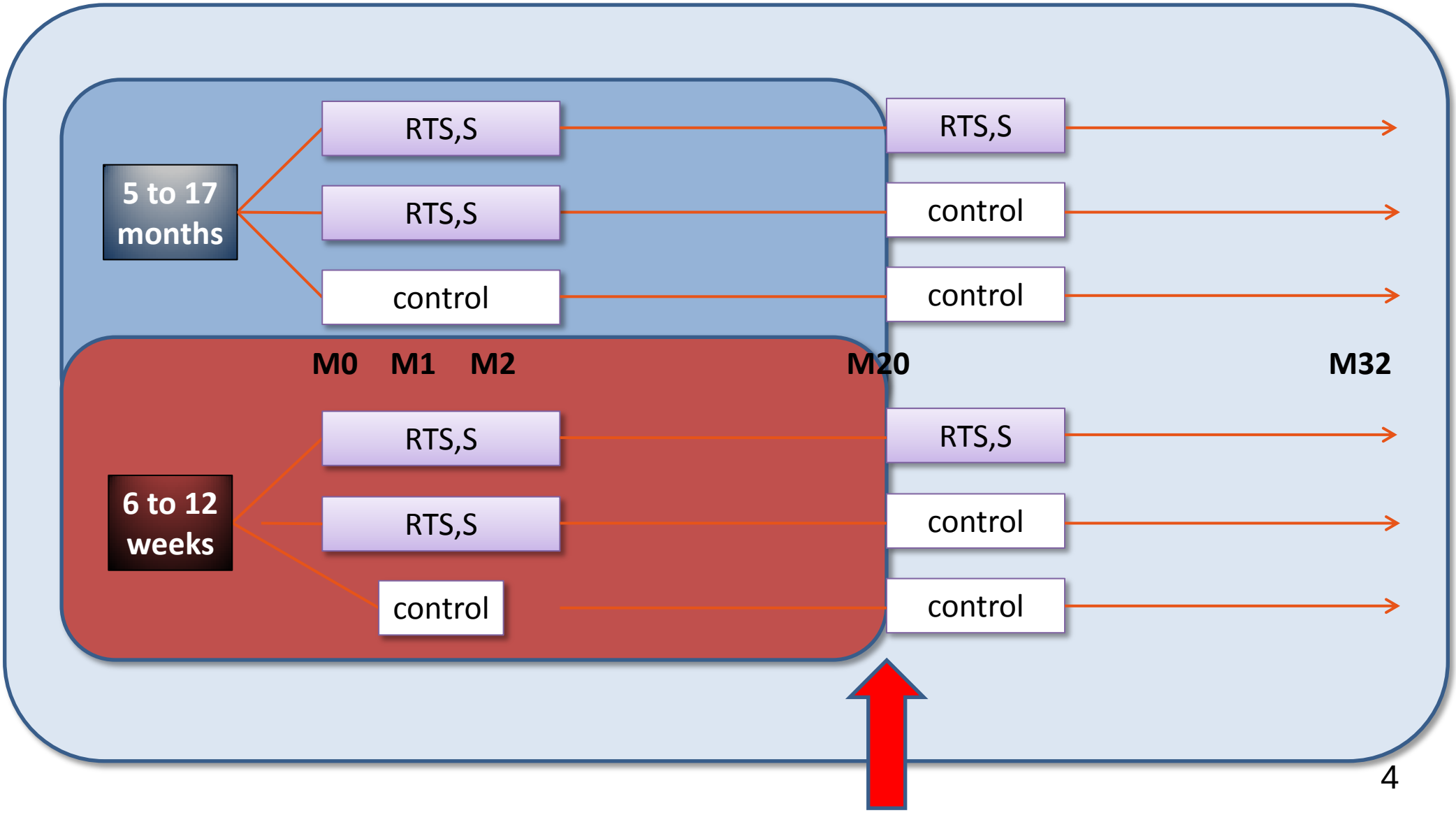
- Chair, Peter Smith
- **Fred Binka (MPAC member)**
- **Kamini Mendis (MPAC member)**
- Malcolm Molyneux
- Paul Milligan
- Kalifa Bojang
- Mahamadou Thera
- Blaise Genton
- Janet Wittes
- Robert Johnson
- Zulfiqar Bhutta (SAGE member)
- Claire-Anne Siegrist (SAGE member)

Observers from European Medicines Agency and National Regulatory Agencies of Kenya, Tanzania, Ghana, Malawi

Pathways for WHO Policy Recommendations on Malaria Vaccines



Design of RTS,S Trial



Vaccine efficacy over 18 months

	VE* in children [95%CI]	VE* in infants [95%CI]
Clinical malaria	**46%** [42 to 50]	**27%** [20 to 32]
Severe malaria	**36%** [15 to 51]	**15%** [-20 to 39]
Malaria hospitalization	**42%** [29 to 52]	**17%** [-7 to 36]
All-cause hospitalization	**19%** [9 to 28]	**6%** [-7 to 17]

- For every 1,000 children/infants, vaccination averted:
  - In children (ITT)**: 37 to 2365** [average: 829] **cases** of **clinical malaria; -1 to 49** [average:18] **cases of severe malaria**
  - In infants (ITT): **-10 to 1402** [average: 449] **cases** of **clinical malaria; -13 to 37** [average: 6] **cases of severe malaria**

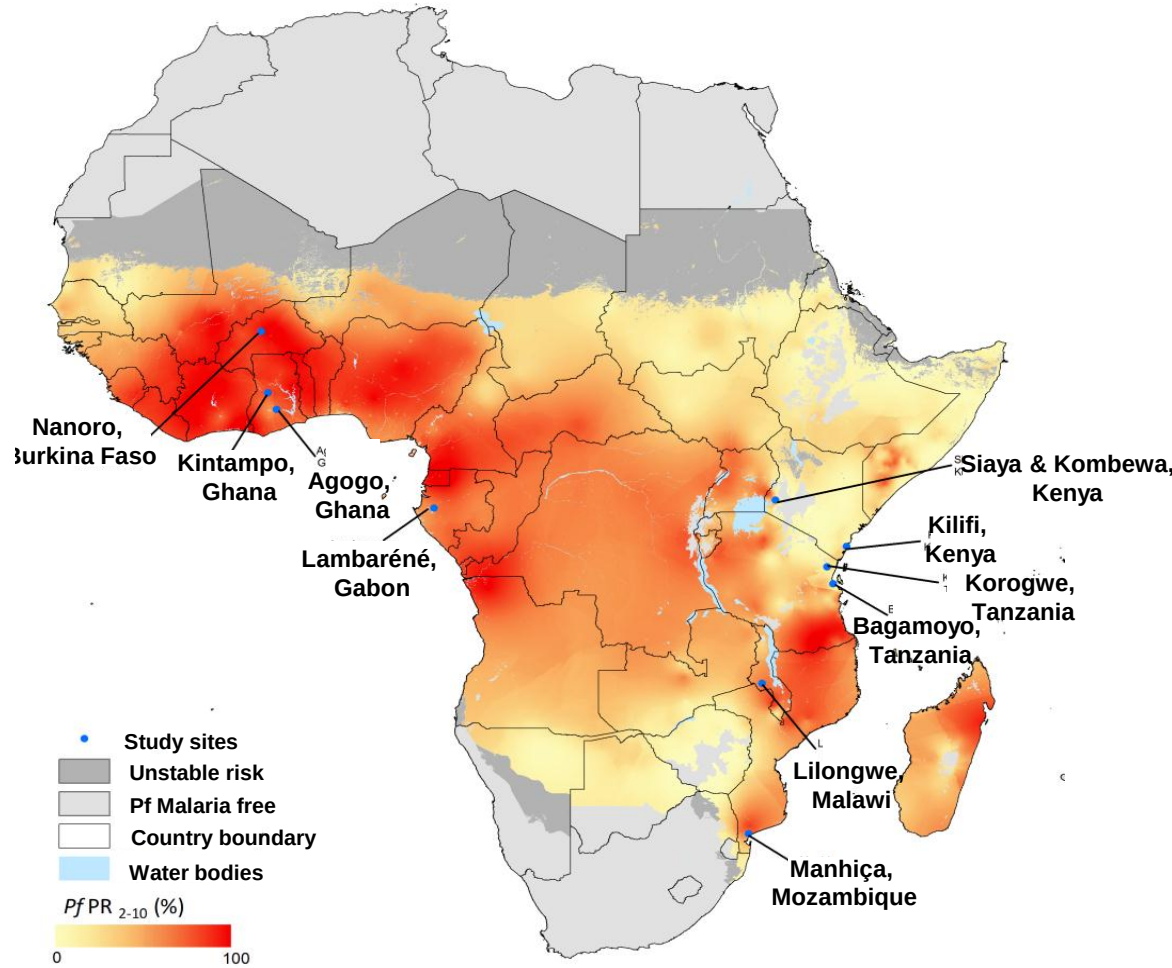
Vaccine efficacy against clinical malaria over 18 months

Time since vaccination	VE* in children [95%CI]	VE* in infants [95%CI]
0-6 months	**68%** [64 to 72]	**47%** [39 to 54]
6-12 months	**41%** [36 to 46]	**23%** [15 to 31]
12-18 months	**26%** [19 to 33]	**12%** [1 to 21]

- Results for 1 year follow-up after booster dose at 18 mo. will be available later in 2014
- Will booster dose restore efficacy to level seen after primary course?
- Will decline in efficacy after booster dose mirror that seen after primary course?
- Will booster dose to those with primary course in infancy bring efficacy up to level of that seen in those who received primary course as child?

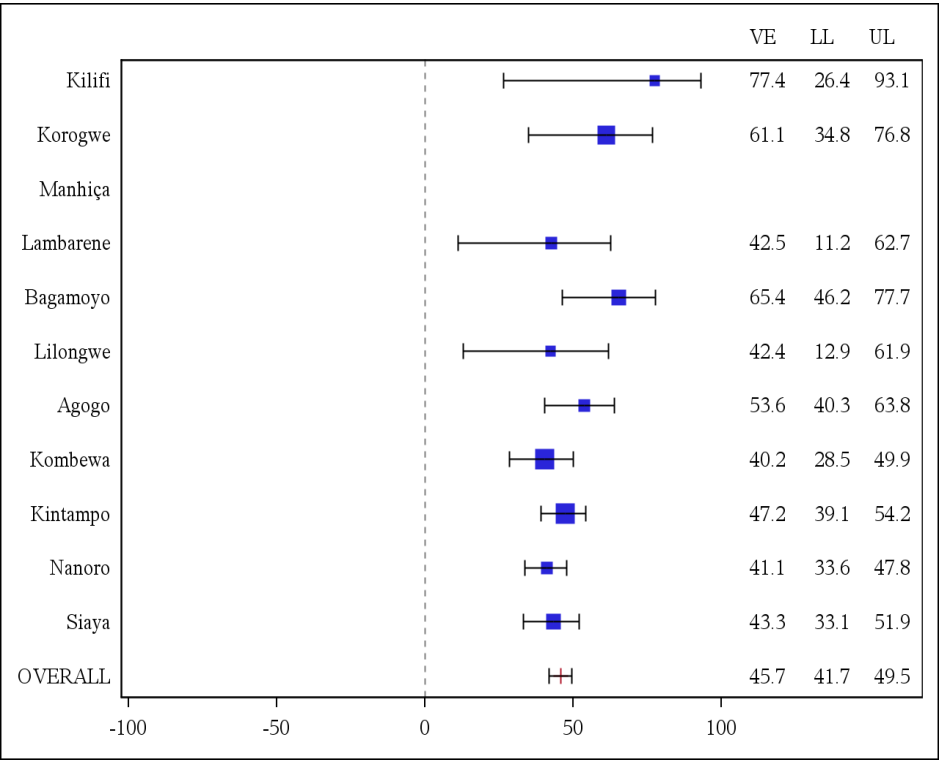
Pivotal Phase III RTS,S malaria vaccine efficacy trial

- Phase 3, randomized, controlled, double-blind trial conducted in
  11 centers in 7 African countries
- 15,460 children enrolled in two age categories:
  - Children aged 5–17 months
  - Infants aged 6–12 weeks
- Co-primary endpoint:
  Vaccine efficacy against clinical malaria during 12 months of follow-up in each age category.
- **Wide range of malaria transmission intensities (0.01 to 2.0 clinical episodes per child per year)**
- **Efficacy measured in presence of other malaria control interventions: 86% ITN coverage in 6-12 weeks and 75% in 5-17 months**

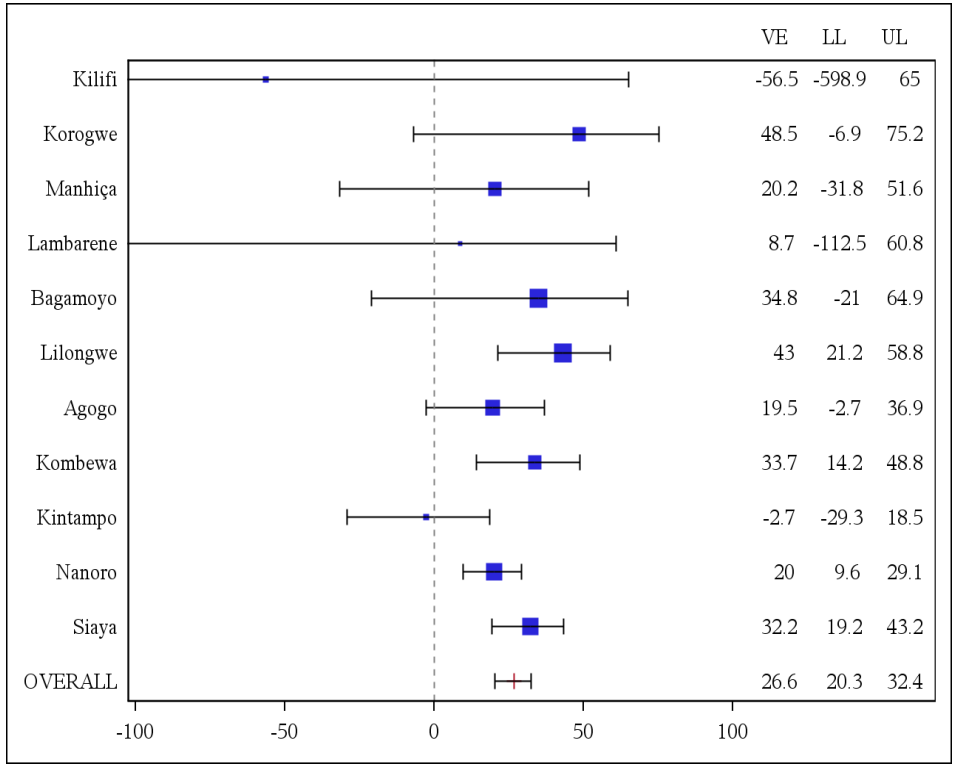


Vaccine efficacy over 18 mo by site – all episodes of clinical malaria

Children 5-17 months



Infants 6-12 weeks



- No clear variation in efficacy according to transmission level.
- Benefit of the vaccine (episodes prevented) likely to be greatest in high transmission settings.
- 3-fold higher immunogenicity for anti-CS IgG in older age group.
  - Immunological immaturity?
  - Interference from maternal antibodies?
  - Interference from co-administration with other vaccines?

Key findings: safety

- No new safety issue has arisen since the previous 2 sets of results from the Phase III trial
- To be assessed by Global Advisory Committee on Vaccine Safety

JTEG Assessment of Results

- In general the efficacy is superior in the 5-17 month age group compared to the 6-12 week age group.
- Efficacy is waning substantially by 18 months, and hence the booster dose data will be important for the policy assessment
- The increased frequency of febrile convulsions and meningitis in vaccine versus control groups will be assessed by the Global Advisory Committee on Vaccine Safety

JTEG Assessment of Results

- If any recommendations for use are proposed by JTEG for SAGE/MPAC decision, it is likely that JTEG will propose a cut-off for the lower limit of transmission below which recommendations for use are not advisable

- JTEG notes that until data are available for VE against infection in a wider population age range, JTEG cannot propose any recommendations for purposes of transmission reduction

JTEG Assessment of Results

- JTEG further noted that any proposed policy recommendations in 2015 will be geographically restricted to sub-Saharan Africa, as no RTS,S data are available from other malaria endemic regions.

- In the scenario that recommendations for use are made, post-licensure district-scale studies appear desirable to better characterise risk/benefit and to allow initial recommendations to be broadened or narrowed through use of a larger dataset than will be available in 2015.

Key analyses expected in 2014

- 30 months follow-up
- Effect of a booster dose at 18 months
- Analyses of the effect of seasonality
- Breakdown of efficacy within 5-17 month age range
- Further analyses as requested by WHO.

Public Health Impact/Cost Effectiveness

- Ongoing work to assess range of predictions between 4 modeling groups, given harmonized inputs.
- Consensus indications of predicted cost-effectiveness of RTS,S/AS01 will be available by time of policy decision
- Policy recommendations will be based on clinical trial data. In some areas a contribution from modeling may be beneficial e.g. guidance for Phase IV design

Key policy question: age group and schedule

- While original target group was infants aged 6,10,14 weeks, the published results raise the question of implementation in children aged 5-17 months
- WHO is commissioning work to model the proportion of malaria hospitalizations “missed” by schedules ending at different ages. Range from DTP3 up to 18 months of age being explored.
- Costing of adding new visits will also be requested in health economic work

Key policy question: role of RTS,S in context of other malaria control measures

- Available data indicates that the demonstrated efficacy is in the presence of a high level of use of insecticide-treated bednets
- Thus any policy recommendations will include wording on continued scale-up of preventive, diagnostic and treatment measures in the context of any RTS,S introduction.

Regulatory, Policy, PQ timings

- Planned EMA filing date June 2014
- Earliest EMA regulatory decision timing is early Q3 2015.
- Tentative MPAC/SAGE date ?Oct 2015 – could be deferred if regulatory timings lengthen
- Possible WHO PQ ?Q1 2016 assuming PQ submission in Oct 2015.

Key Messages from WHO

- Detailed Q&As available on WHO website
- RTS,S/AS01 will be evaluated as an addition to, not a replacement for, existing preventive and treatment measures
- Too early to draw conclusions about the public health role of RTS,S/AS01
- Depending on the results expected in 2014, and on the regulatory submission timings, WHO will make the first malaria vaccine policy recommendations in late 2015.

Updating the Guidelines for the Prevention and Treatment of Malaria

MPAC meeting
WHO HQ, 14 March 2014

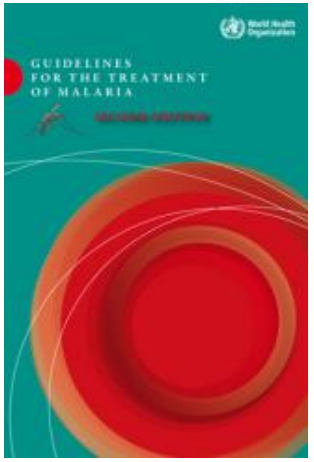
Prof. Nick White
Co-chair, Chemotherapy Technical Expert Group

WHO Guidelines for the Treatment of Malaria (MTGs)

- The WHO Guidelines for the Treatment of Malaria (MTGs),
  - provide comprehensible, global and evidence-based guidelines for the formulation of policies and protocols for the treatment of malaria.
  - was first published in 2006 and a revised edition (2nd edition) published in 2010.
  - is available in hard and web-based versions.
  - the current edition of which is available in English, French and Spanish.

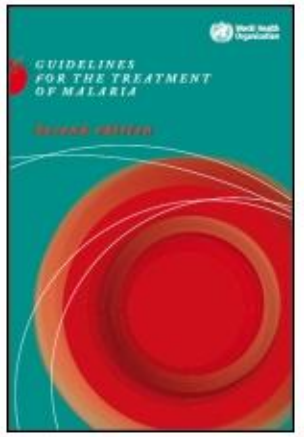
Target audience

- primarily policy-makers in ministries of health, who formulate national treatment guidelines.
- in addition, the other groups working in public health and institutions should also find them useful



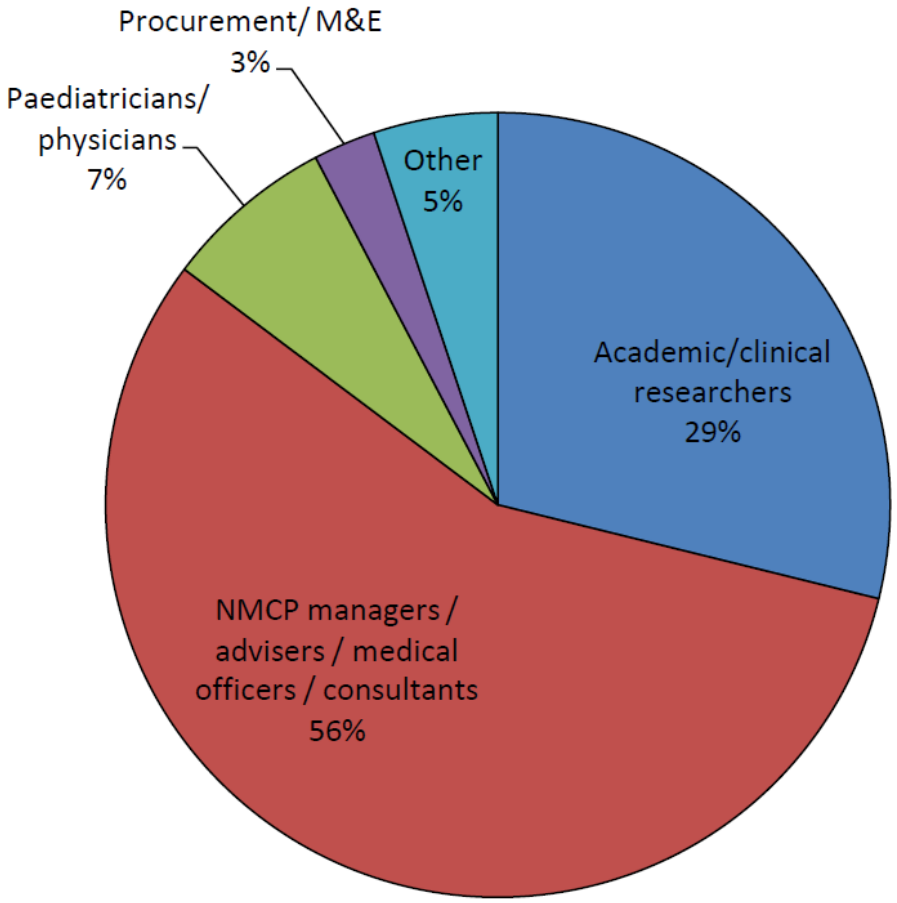
Malaria Treatment Guidelines - process

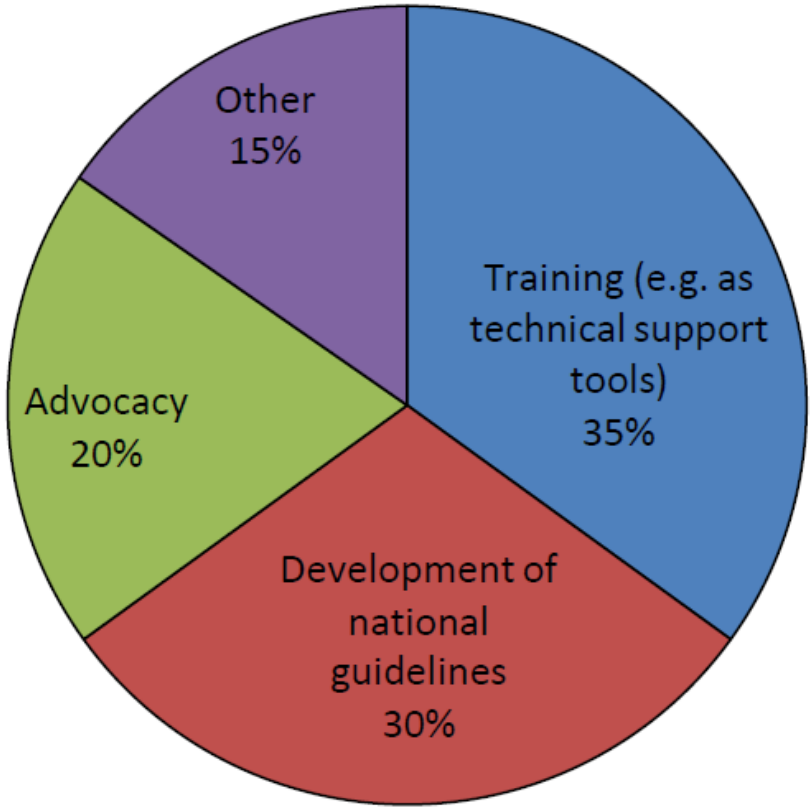
- Scoping meeting to define areas for review – *Feb 2013*
- Commission of reviews of available evidence – *May 2013*
- Proposal for MTG review approved by WHO Guideline review committee – *July 2013*
- Completion of the systematic reviews and Grade tables *November 2013*
- TEG meeting to review and reach consensus on the draft recommendations *(5-8 November 2013)*
- Dosing working group *(Q1-2 2014)*
- Final wrap up meeting *(Q2-3 2014)*
- External and internal review *(Q3 2014)*
- Final clearance through the WHO GRC and other WHO in-house processes *(Q3-4 2014)*



User survey

Majority of respondents thought the guidelines were clearly written, appropriate in scope and size, and practically very useful. Voted to retain current format and preserve the evidence base in the main document.



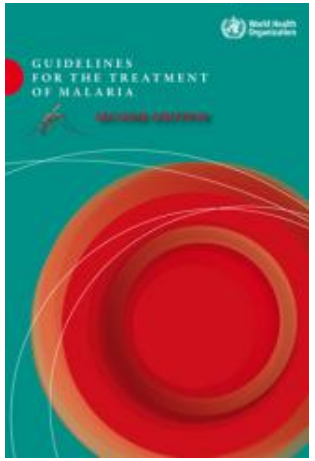


What has changed in the 2014 guidelines?

- **No major changes in ACT recommendations.**
- **Antimalarial treatment of severe malaria unchanged.**
- **Acceptance that the target of dosing is equivalent exposure across all age groups and patient sub-groups**

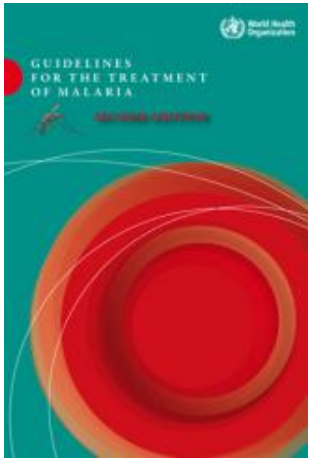
-Formation of dosing sub-group to evaluate and model all available PK information and formulate revised dosing recommendations.

- Parenteral artesunate, DHA-piperaquine.



What is new in the 2014 guidelines?

- **Reviews**
  - Safety and effectiveness of mass drug administration
  - Safety and efficacy of 0.25 vs. 0.75mg/kg primaquine as gametocytocidal agent for *P. falciparum*
  - Role of RDTs in malaria elimination programmes
- **New chapters/sections**
  - Intermittent preventive treatments (IPTp; IPTi)
  - Seasonal malaria chemoprevention
  - Chemoprophylaxis in travelers
  - Section on treatment of artemisinin resistant falciparum malaria



Update on TEG activities and antimalarial drug resistance

MPAC meeting
WHO HQ, 14 March 2014

Dr. Pascal Ringwald
Coordinator Drug Resistance and Containment

World Health Organization

GLOBAL MALARIA PROGRAMME

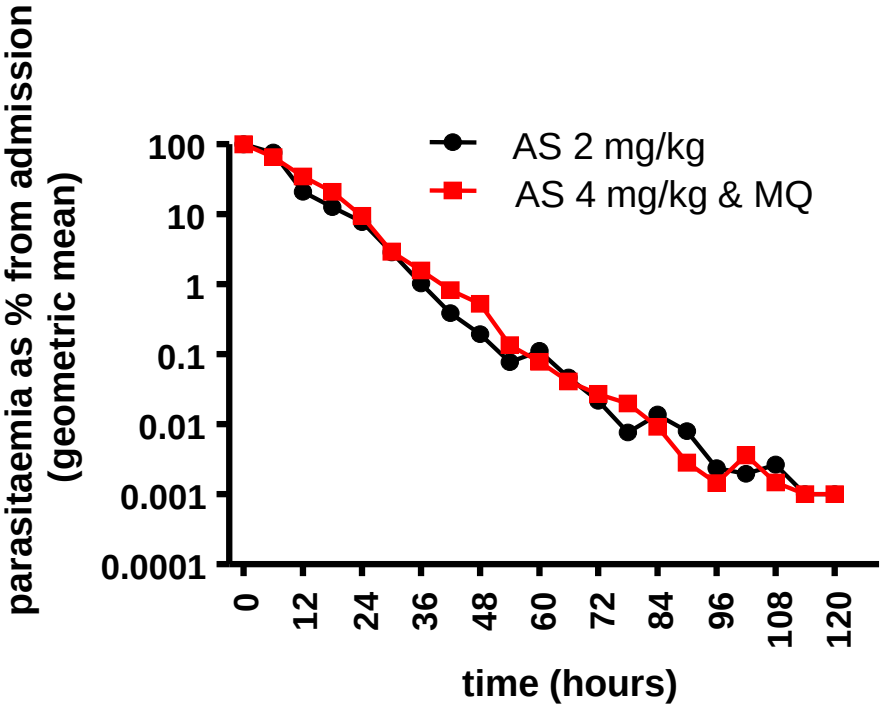
Next TEG meeting

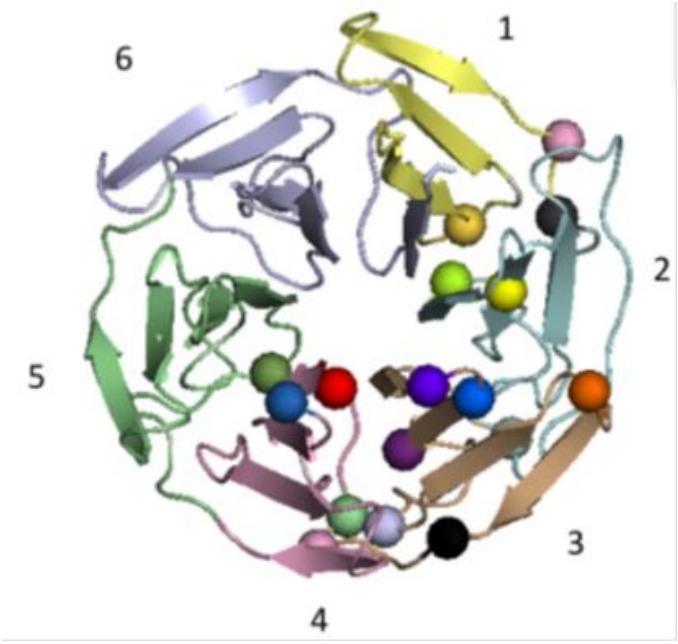
In Geneva 28-30 April 2014

- Update on drug resistance
- Modelling
- Update on recent containment and elimination efforts
- Global Technical Strategy

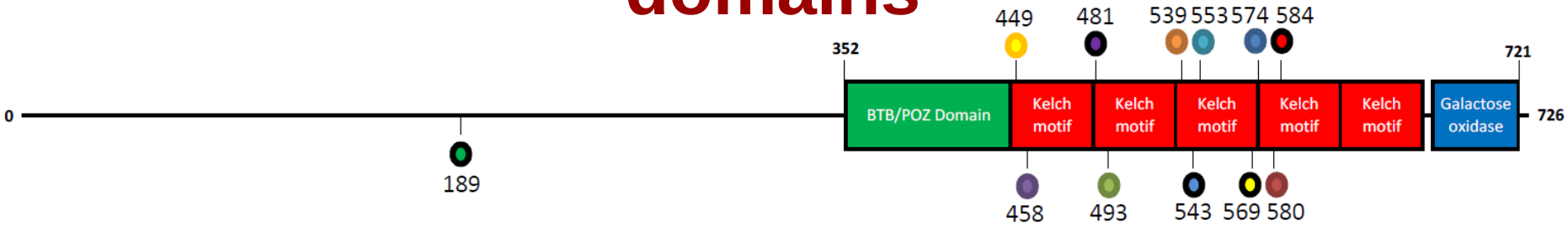
Surveillance: major public health outcomes

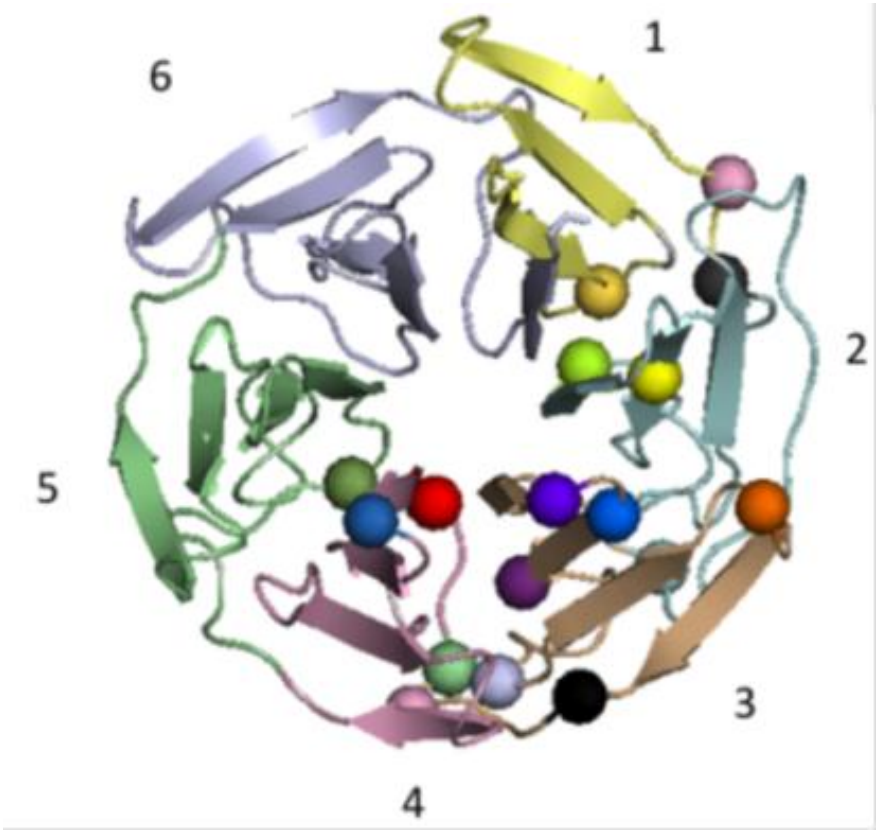
- Collaboration between NMCP, research institutes and WHO led to the identification of a molecular marker associated with delayed parasite clearance in patients treated with artemisinin.



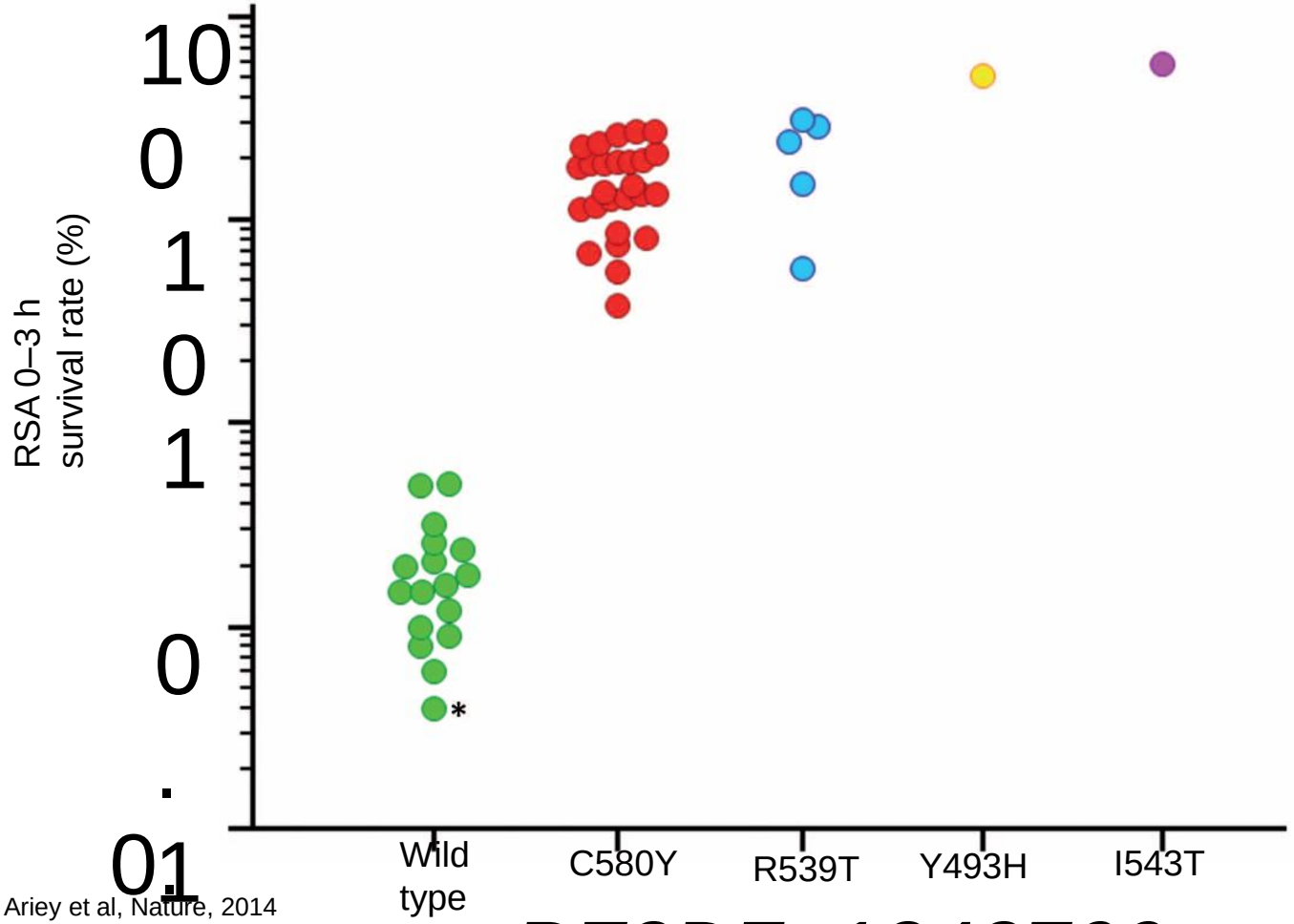


Most K13 SNPs reside in the “propeller” domains





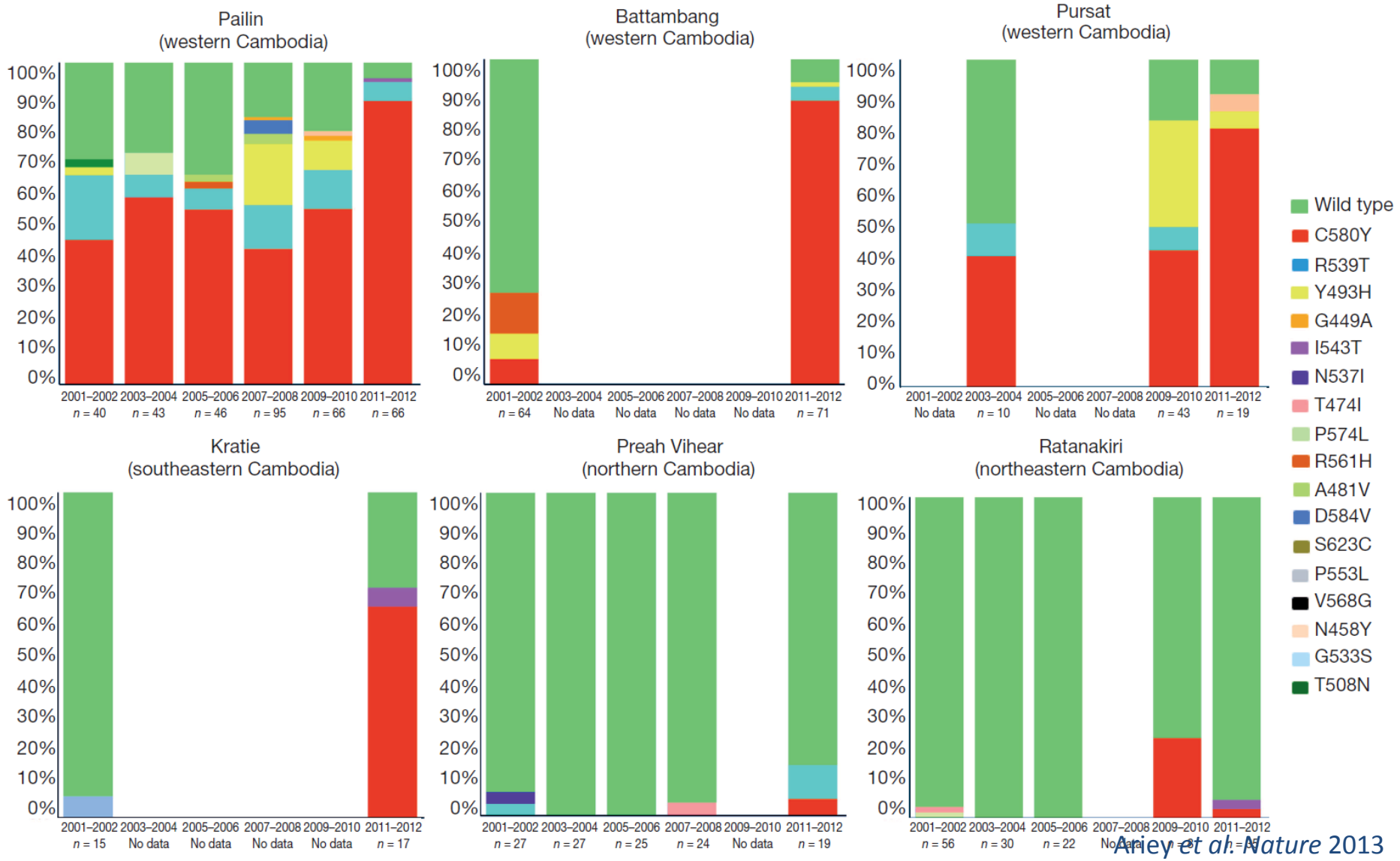
Survival rates of Cambodian parasite isolates in the RSA0–3 h, stratified by K13-propeller allele



Frederic Ariey et al, Nature, 2014

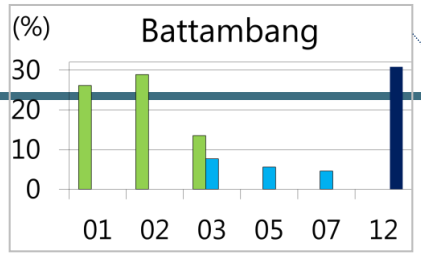
PF3D7_1343700 polymorphisms

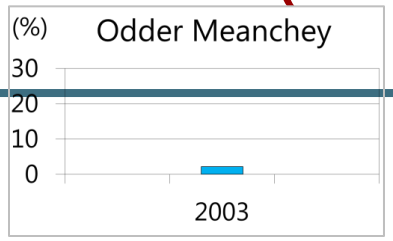
Frequency of K13-propeller SNPs in 886 parasite isolates in six Cambodian provinces in 2001–2012

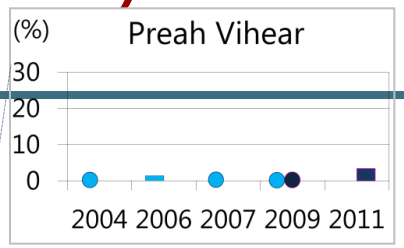


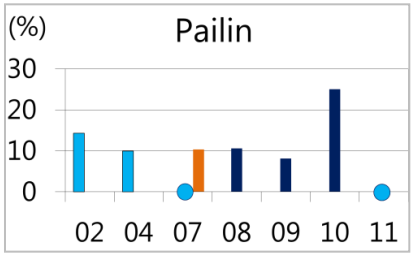
Ariey *et al*. *Nature* 2013

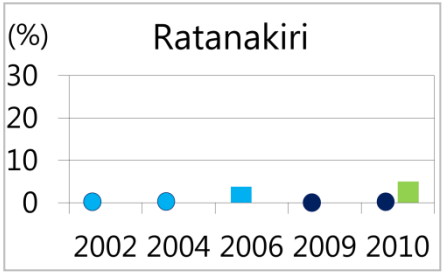
Treatment failure rates after treatment with an ACT, Cambodia (2001–2012)

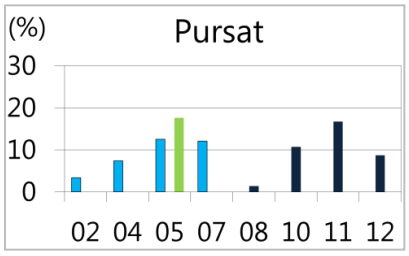


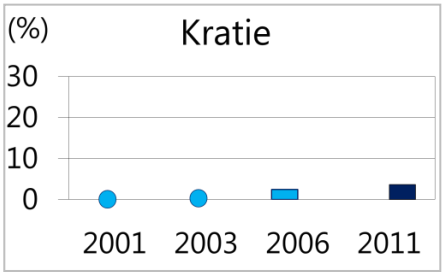


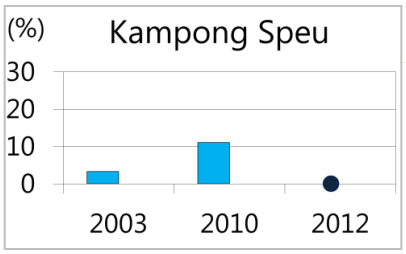


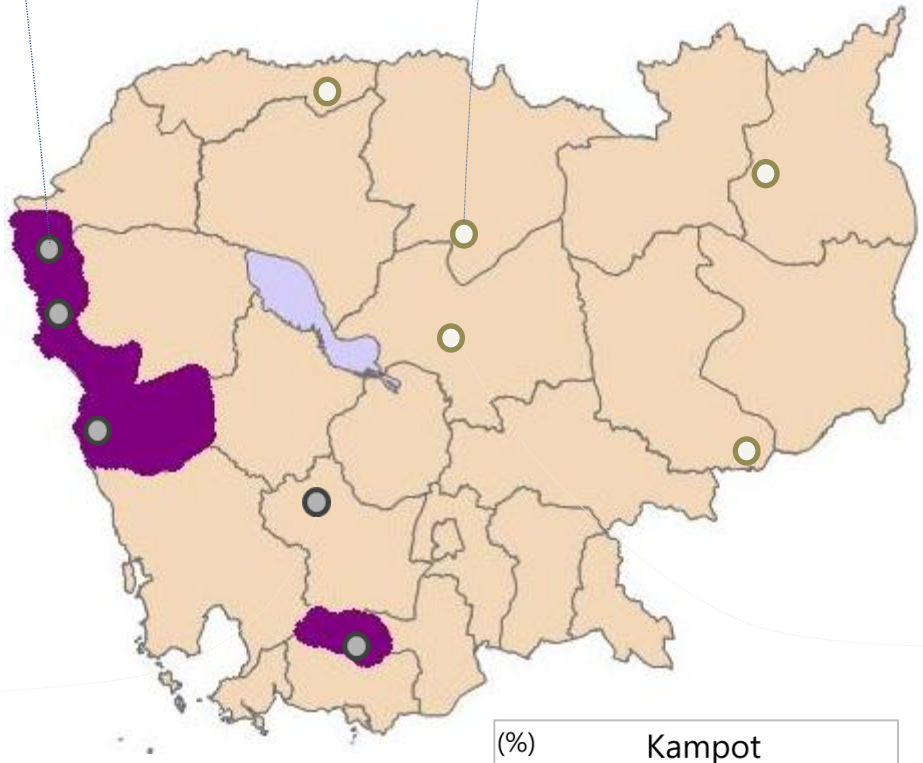




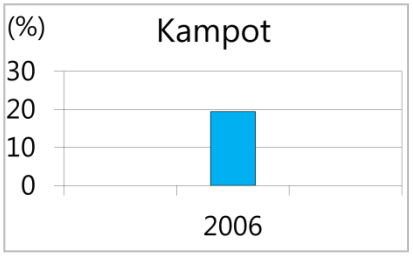


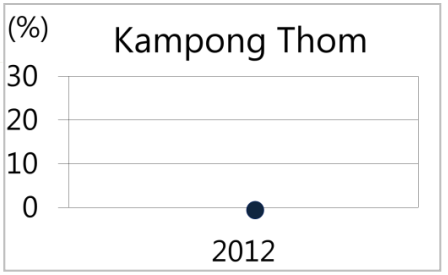






artemisinin resistance containment activities

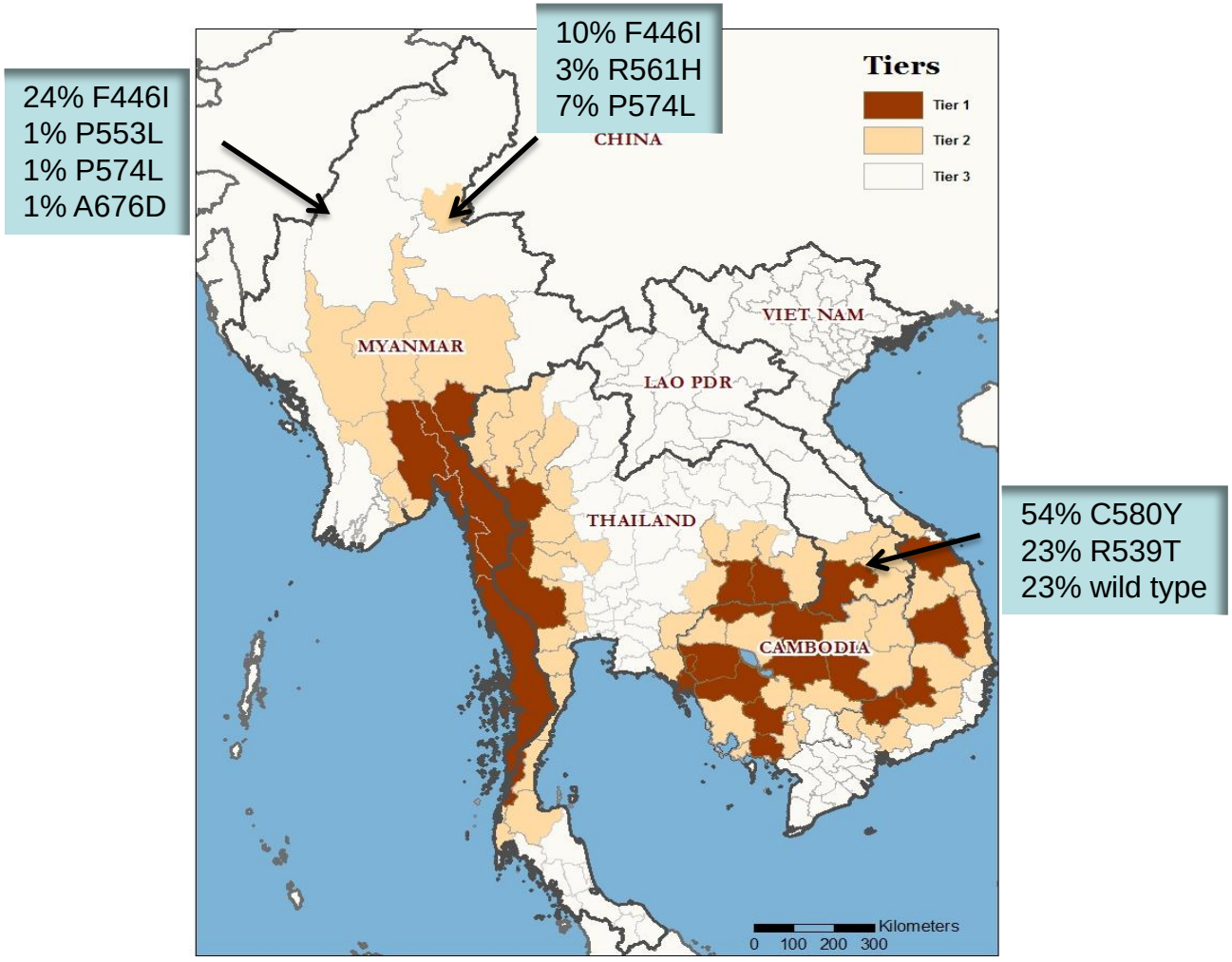




Confirmatory study in Suriname

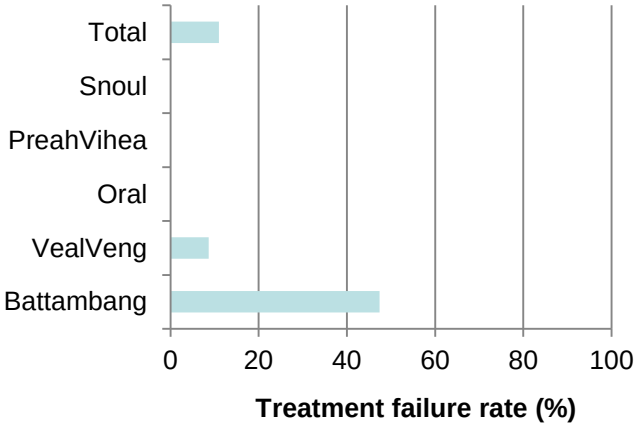
- Study started on July 2013
- 35 patients enrolled (Fr. Guyana: 29 patients)
- All Patients, except 3 (10 %), had negative slides at 72 hours after the first dose of treatment.
- Follow up until day 28: 7 (All ACPR)

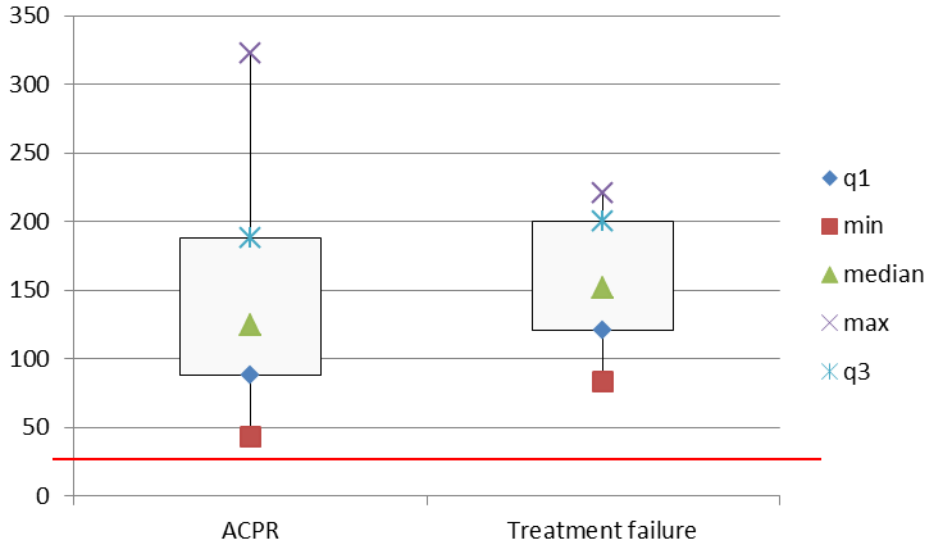
Surveillance: review of the data by the TEG



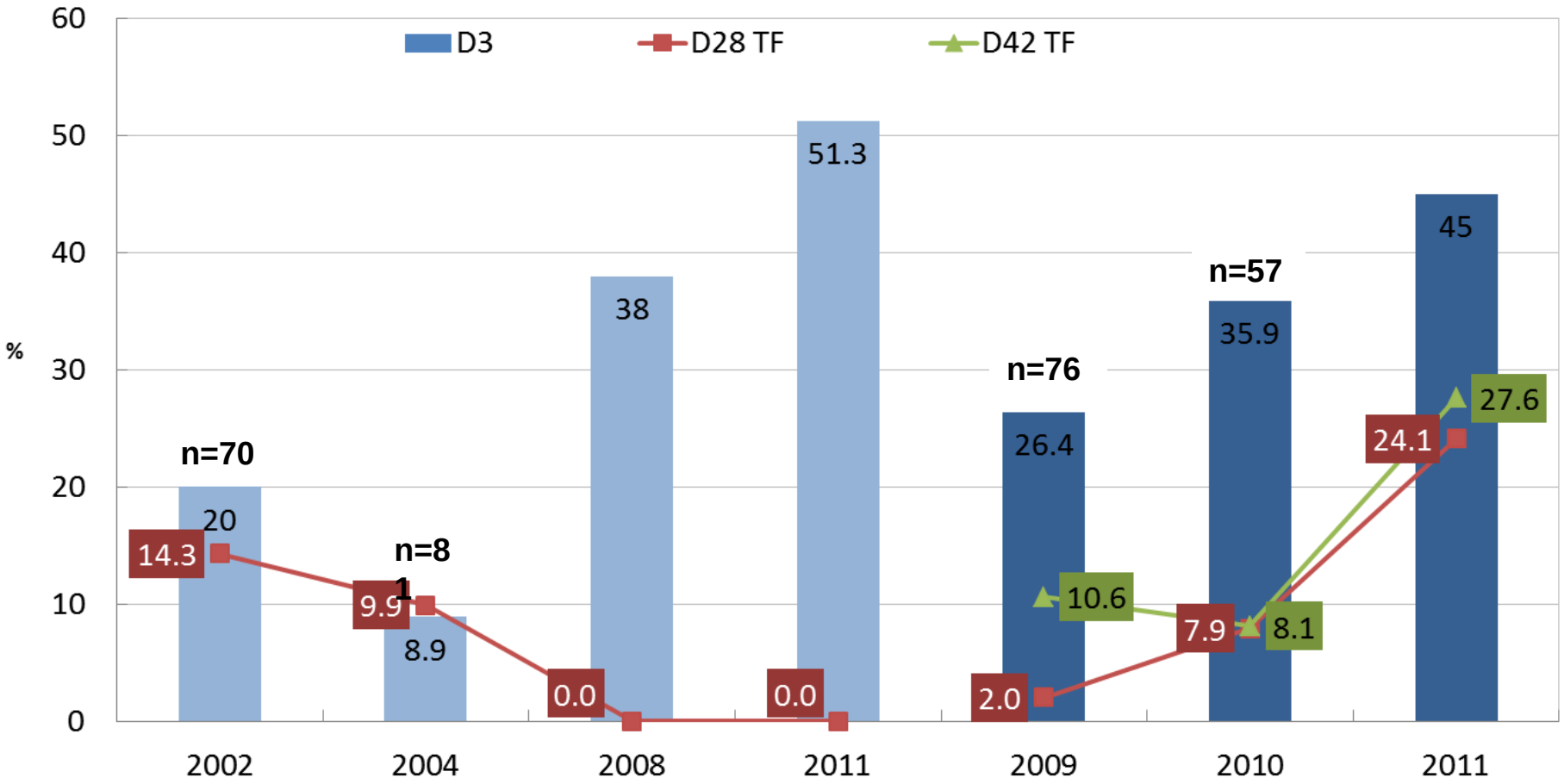
Drug Resistance in Cambodia

- Atovaquone-proguanil
  - Currently 1st line treatment for falciparum malaria in Pailin province;
  - After 7 months 5% of parasites tested in Pailin harboring 268 *cyt b* mutation *(CNM/Pasteur, unpublished data);*
  - This rapid loss to resistance is very likely to be repeated elsewhere.
- Piperaquine
  - Blood concentration of piperaquine on day 7 collected in 2011 and 2012 (n = 94; TF = 11; ACPR = 82)





ACT efficacy in Pailin, Cambodia (2002-2011)



artesunate-mefloquine

dihydroartemisi

World Health Organization

GLOBAL MALARIA PROGRAMME

New treatment policy in Cambodia

- Artesunate-mefloquine (co-formulated) in 4 provinces with high DP treatment failure (Battambang, Oddar Meanchey, Pailin and Pursat);
  - under tight (monthly) monitoring of molecular markers (*pfmdr1* copy numbers, K13). If *pfmdr1* copy numbers elevated: artesunate-pyronaridine or artesunate-atovaquone-proguanil;
- Dihydroartemisinin-piperaquine in the other provinces;
- Pyramax as implementation research study for all patients except for small children (<20kg), pregnant women and people who cannot be enrolled for other reasons;
- DOT in 100% cases, hospitalization.

Update on Malaria Burden Estimation ERG Recommendations *and* Surveillance, Monitoring and Evaluation Technical Expert Group (SME TEG)

MPAC meeting
WHO HQ, 14 March 2014

Dr Richard Cibulskis
Strategy, Economics and Elimination

World Health Organization

GLOBAL MALARIA PROGRAMME

Malaria Burden ERG Recommendations: Cases

1. For the 2014 WMR, WHO should use MAP's case estimates from the "cube" for African countries without strong surveillance systems.

   MAP "cube" outputs will be used to produce estimates for SSA - first draft expected July/August for review by SME TEG.

2. WHO should discuss with partners to determine the feasibility of collecting prevalence data through MIS on all age groups, not just 6-59 month age group.

   Issue raised at MERG in context of surveillance in changing transmission settings. Can be brought to SME TEG.

3. The analysis examining parasite prevalence stratified by type of care-seeking behavior should be supplemented with more recent surveys and surveys from outside of Africa.

   Work is ongoing

4. As with cases and deaths, WHO should show the reported country-level parasite prevalence alongside the modeled parasite prevalence in the WMR 2014.

   Will be done

Malaria Burden ERG Recommendations: Deaths

1. Using the STPH model, WHO should calculate deaths by the same age groups as IHME and Nick White, and compare the results.

   Awaiting data sets

2. Reach out to 10 hospitals in endemic areas to determine whether they are willing to share their data on the age distribution of severe malaria. The goal is to develop a list of hospitals for adult malaria mortality research.

   Malcolm Molyneux sent a letter to 10 mid-size hospitals in malaria endemic countries, inquiring about the availability of admissions and parasitological diagnosis data.

3. Assembled all available data to examine adult death from malaria. This will include a literature search for hospital and other studies, including RTS,S trial data when made available.

   WHO has conducted a literature review of studies that include verbal autopsy-classified adult malaria deaths.

   Tom Smith (STPH) is working on a meta-analysis of the EIR-mortality relationship in MTIMBA datasets. This analysis will be used in conjunction with an analysis of the relationship between prevalence and EIR to help account for case management in the WHO malaria mortality estimates.

Malaria Burden ERG Recommendations: Deaths

1. In an attempt to validate the InterVA methodology, a sample of INDEPTH records should be sent to Malcolm Molyneaux to determine whether, based on his field experience in hospital, he would code the deaths the same.

   Awaiting response

2. A draft protocol for a study using hospital data on mortality and RDT results should be drafted and circulated to the ERG for comment.

   Awaiting response

   WHO should maintain the same methodology for the World Malaria Report malaria mortality estimates until further research is conducted.

   Plan to use MAP cube in CHERG estimates and present results to SME TEG

4. WHO and the malaria community should consider eliminating the over-five/under-five dichotomy; the message is confusing because “over-five” is often termed “adult".

Responsibilities of SME TEG

Provide advice to WHO on SME at national, regional and global level:

a) choice of indicators for monitoring the financing, coverage, quality and impact of malaria control interventions at national and global level;

b) strategies for obtaining, synthesizing and disseminating information on the indicators globally, including modeled estimates of intervention coverage and disease burden;

c) guidance that WHO provides on (i) surveillance of infections, cases and deaths and the use of the data in decision-making, (ii) establishing systems for monitoring programme financing and coverage, (iii) evaluating the impact of malaria interventions and programmes.

d) evaluation of the accuracy and integrity of SME data at the national, regional and global level;

e) approaches for strengthening the capacity of member states to generate and use key information

f) identification of gaps in evidence and suggesting priority research areas in the field of SME.

Composition of SME TEG: areas of expertise

- Monitoring finances
- Monitoring vector control
- Monitoring preventive therapies
- Monitoring diagnosis and treatment
- Measurement of morbidity and mortality
- Tracking progress of elimination

- Health information systems
- Household surveys
- Health facility surveys
- Demographic surveillance systems

- Members working in NMCPs

- MPAC members

Currently 18 members selected

Ways of working

- Up to two meetings per year: February & August
- Membership: initial term of up to three years, renewable once
- Invited observers - key partners
- Call on specific expertise for certain issues
- Working with MERG
  - SME TEG discussed at MERG in June 2013 and MERG in Feb 2014
  - WHO continued involvement with MERG (but no longer co-chair)
  - SME TEG and MERG memberships overlap
  - SME TEG to share recommendations, draft documents with MERG
  - MERG used as filtering mechanism for issues that SME TEG should consider
  - look to MERG to help implement recommendations

Evidence Review Group on Malaria Diagnostics in Low Transmission Settings: meeting report and draft recommendations for MPAC consideration

ERG meeting held in Geneva, Switzerland
16-18 December 2013

MPAC meeting
WHO HQ, 14 March 2014

Participants

- **Co-Chairpersons:** Brian GREENWOOD, Kevin MARSH
- **Rapporteur:** Ruth ASHTON
- **ERG members:** Michael AIDOO, Philip BEJON, David BELL, Teun BOUSEMA, Lydie CANIER, Qin CHENG, Peter CHIODINI, Jackie COOK, Valerie D'ACREMONT, Chris DRAKELEY, Iveth GONZALEZ, Mallika IMWONG, Edwin KAMAU, Paul LABARRE, Sylvia MEEK, Ivo MUELLER, Nyasatu NTSHALINTSHALI, Lucy OKELL, Christian F. OCKENHOUSE. André Lin OUEDRAOGO, Mark PERKINS, Georges SNOUNOU, Venkatachalam UDHAYAKUMAR
- **Observers:** Melto J. ELIADES, Heidi HOPKINS, Kathy TIETJE
- **WHO Secretariat:** Hoda ATTA, Andrea BOSMAN, Eva CHRISTOPHEL, Jane CUNNINGHAM, Josephine NAMBOZE, Robert NEWMAN, Piero Luigi OLLIARO, John REEDER, Pascal RINGWALD, Vaseeharan MOORTHY

Meeting procedures described on page 4 of the Report

Meeting objectives

P. falciparum & P. vivax

- Review current knowledge on contribution of asymptomatic parasitaemia to transmission, particularly in areas with low transmission
- Review diagnostic performance, technical and resource requirements of available nucleic acid amplification (NAA) methods for diagnosing low density infections with sexual and asexual malaria parasites; recommend most suitable methods for use in population surveys and active or reactive investigations
- Review requirements to build capacity and ensure quality for NAA methods to support programmatic interventions in pre-elimination and elimination settings
- Review and suggest revisions to current WHO recommendations for malaria diagnostic approaches in low transmission settings
- Review malaria diagnostic R&D pipeline; reach consensus on preferred product characteristics of new diagnostic tools to meet public health needs for malaria elimination

Focus is NOT High/moderate transmission settings or role of NAA based tests for detection of clinical cases

Table 1 Malaria surveillance in different transmission settings and phases of control

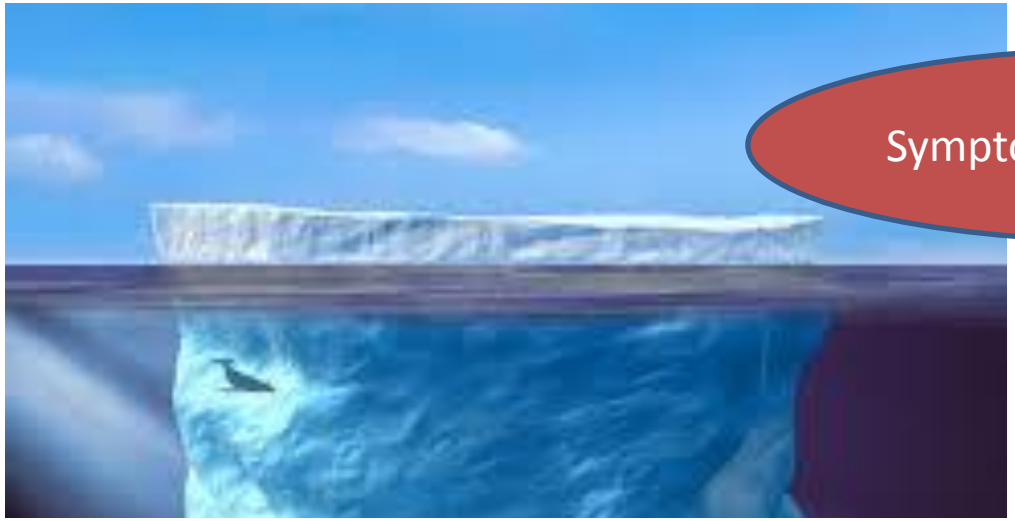
	Control phase		Elimination phase
Transmission:	High & moderate	Low	Very low
Parasite prevalence (2-9 yrs):	>10%	<10%	
Incidence:	Cases and deaths common and concentrated in <5yrs	Cases and deaths less common distributed according to mosquito bite exposure	Cases sporadic
	Limited temporal variation	Variable within and between years Risk of epidemics	Imported cases may be high proportion of total
	Limited geographical variation	Geographical heterogeneity Concentrated in marginal populations	Focal distribution
Fevers:	Proportion of fevers due to malaria relatively large	Proportion of fevers due to malaria small	Proportion of fevers due to malaria very small (though may be high in certain foci)
Health facility attendance:	High proportion due to malaria	Low proportion due to malaria	
Vectors:	Efficient	Controlled efficient/ inefficient	Controlled efficient/ inefficient
Aims of programme:	Mortality & case reduction	Case reduction	Eliminate transmission
Surveillance system			
Resources:	Low expenditures per head Low quality and poor accessibility of services	Widespread availability of diagnostics and treatment	Resources to investigate each case
Data recording:	Aggregate numbers	Aggregate numbers Lists of inpatients and deaths → lists of all cases	Case details
Investigation:	Inpatient cases	Inpatient cases → all cases	Individual cases

Settings characterized by :

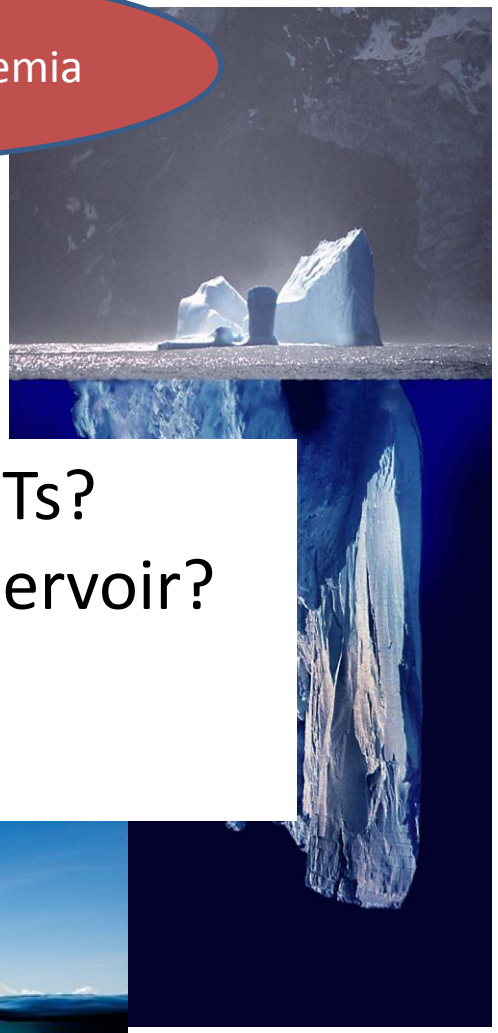
- Lower incidence of confirmed cases
- More uniform spread by age or concentrated in population groups with higher exposure / focal within districts
- Lower mortality rates
- Parasite prevalence (2-9yrs): <10%

High coverage of interventions:
diagnostic testing
LLIN, IRS
Surveillance system with good reporting rates in place

Source: WHO, 2012. Disease surveillance for malaria elimination: an operational manual.



Symptomatic parasitemia



Who are we missing with microscopy and RDTs?
What factors influence the asymptomatic reservoir?
What is its contribution to transmission ?
When and how to target it ?

Asymptomatic parasitemia

Targeting the asymptomatic reservoir

- Multiple survey approaches utilize diagnostic testing
  - Prevalence surveys, focal screen and treat (FSAT), mass screen and treat (MSAT), highly focused screening and treatment (HiFSAT) – **this ERG meeting did NOT analyse and recommend specific survey approaches vs others**
- Diagnostic tools for infection detection
  - Microscopy, RDTs, nucleic acid based methods
  - Sexual vs asexual parasites; hypnozoites
  - Considerations : 'required' limit of detection for public health impact, quantitative vs non quantitative, programmatic suitability, cost, and quality control
  - Role of serology
- Knowledge gaps and research needs

Responding to questions from countries

- *What are the recommended tests to detect asymptomatic infections in population surveys, active case detection, screening, and case management in elimination settings?*
- *What is the gold standard of malaria diagnosis in elimination setting ?*
- *What are the recommended diagnostic tools to be used at community level in areas targeted for malaria elimination, considering the limitations of microscopy and RDTs?*
- *What is the role of PCR in malaria elimination settings for surveillance and case management, and what are the requirements for quality assurance?*
- *What are the most sensitive and easy to use assays to detect gametocytes and their contribution to transmission, for use in research studies ?*
- *What is the best screening tool for detection of malaria asymptomatic carriers in airports and at borders?*
- *Can current serological tests (ELISA) assist in differentiating recent versus old infection?*
- *What are the best diagnostic tools to confirm interruption of transmission, for certification of malaria elimination?*
- *What resources and tools are required to sustain diagnosis capacity in low transmission settings and/or in areas at risk of re-introduction of malaria?*

ANNEX 1 of the Report (pages 23-24)

Collated from Malaria Regional Advisors EMRO. AFRO, PAHO

Inform/update current recommendations/guidance

ANNEX 2 of the Report (pages 25-29)

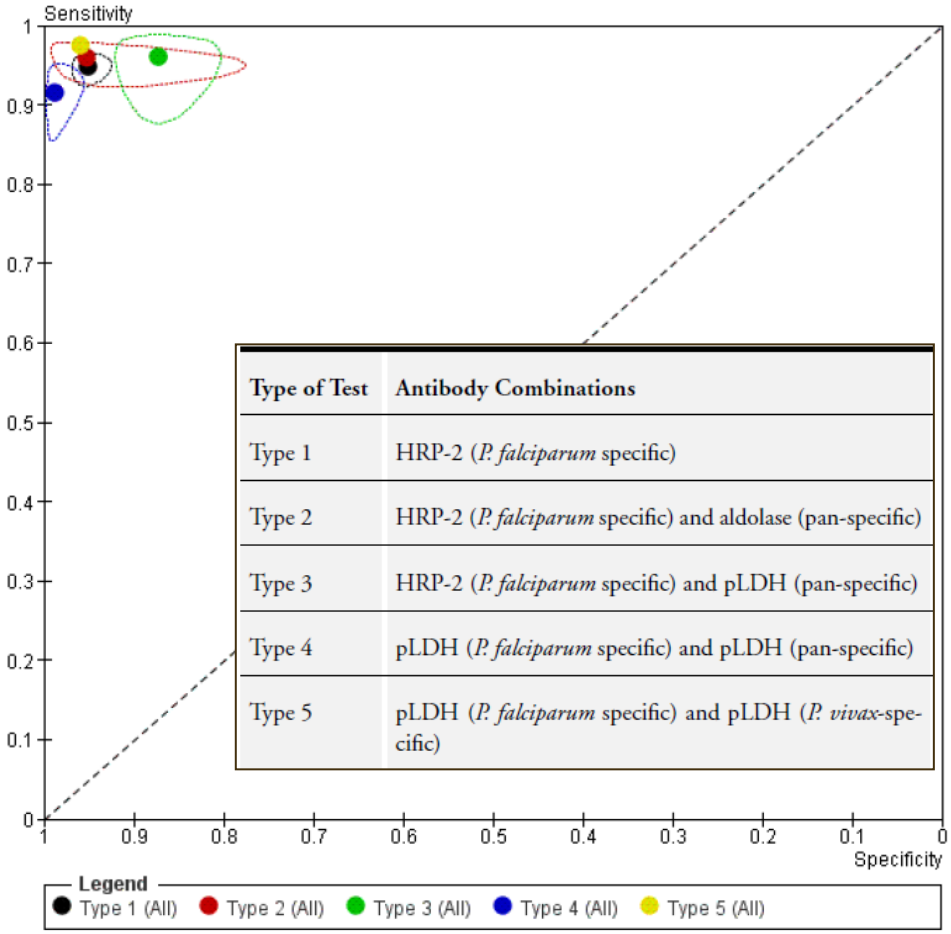
Draft Recommendations

1

- *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.*

Cochrane systematic review of RDTs

- Review of 94 unique eligible studies comparing RDTs with microscopy:
  - For HRP-2, the meta-analytical average sensitivity and specificity (95% CI) were 95.0% (93.5% to 96.2%) and 95.2% (93.4% to 99.4%), respectively.
  - For pLDH, the meta-analytical average sensitivity and specificity (95% CI) were 93.2% (88.0% to 96.2%) and 98.5% (96.7% to 99.4%), respectively.



Type of Test	Antibody Combinations
Type 1	HRP-2 (*P. falciparum* specific)
Type 2	HRP-2 (*P. falciparum* specific) and aldolase (pan-specific)
Type 3	HRP-2 (*P. falciparum* specific) and pLDH (pan-specific)
Type 4	pLDH (*P. falciparum* specific) and pLDH (pan-specific)
Type 5	pLDH (*P. falciparum* specific) and pLDH (*P. vivax*-specific)

Abba K, Deeks JJ, Olliaro P, Naing CM, Jackson SM, Takwoingi Y, Donegan S, Garner P. Rapid diagnostic tests for diagnosing uncomplicated *P. falciparum* malaria in endemic countries. *Cochrane Database of Systematic Reviews* 2011, Issue 7. Art. No.:CD008122. DOI: 10.1002/14651858.CD008122.pub2.

Draft Recommendations

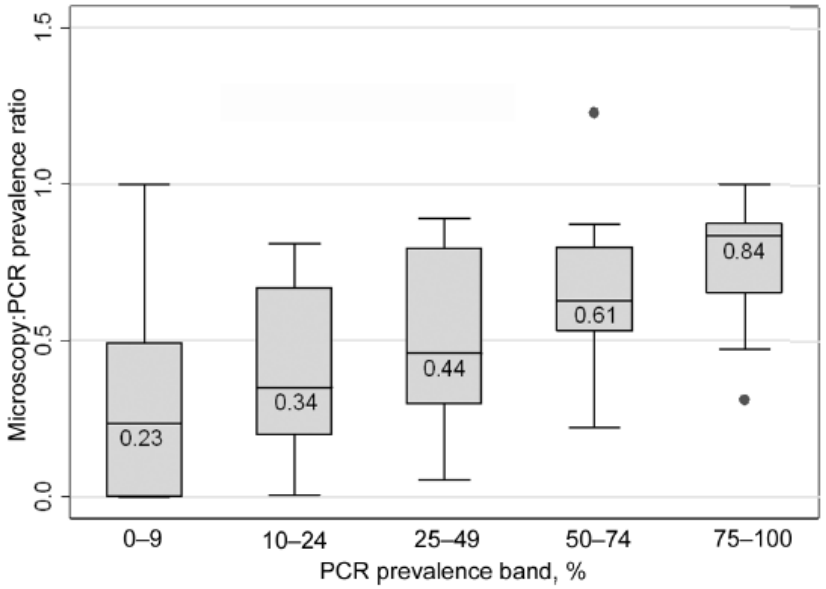
- *Generally, the use of more sensitive diagnostic tools should be considered only in low transmission settings where there is already widespread implementation of malaria diagnostic testing and treatment and low parasite prevalence rates (e.g. < 10%). Use of nucleic acid amplification (NAA)-based methods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

Draft Recommendations

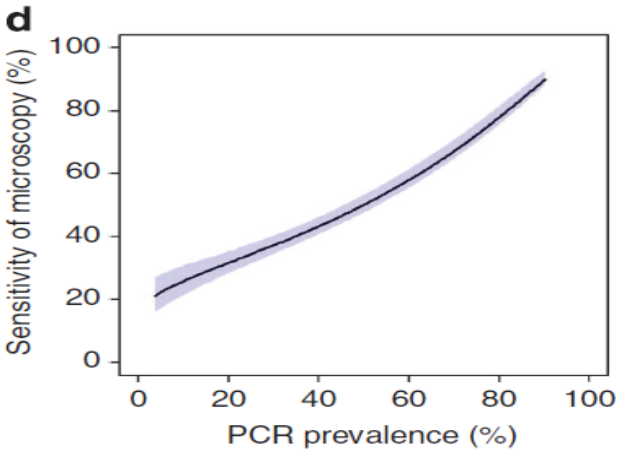
- *Submicroscopic Plasmodium falciparum and P. vivax infections are common in low as well as high transmission settings. A number of nucleic acid amplification techniques are available and are more sensitive in detection of malaria compared to RDTs and microscopy. The use of NAA methods by malaria programs should be considered for surveys aimed at mapping prevalence of malaria, including submicroscopic infections, and to increase the power of surveys at low transmission intensity.*

Submicroscopic *P. falciparum* infection

Okell C, et al. JID 2009: 200: 1509-17



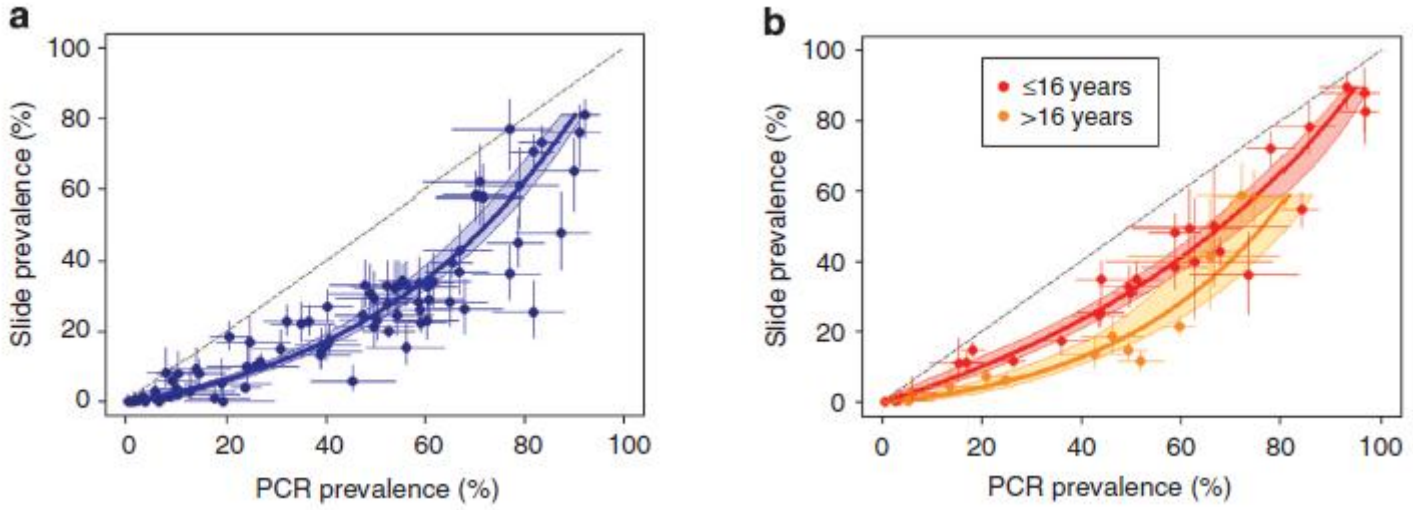
Okell C, et al. NC 2012: 3:1237
DOI: 10.1038/ncomms2241



A low percentage of total infections is detected by microscopy in areas of low transmission, compared with areas at high transmission, (12.0% when at PCR prevalence was <10% versus 74.5% at PCR prevalence >75%).

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.

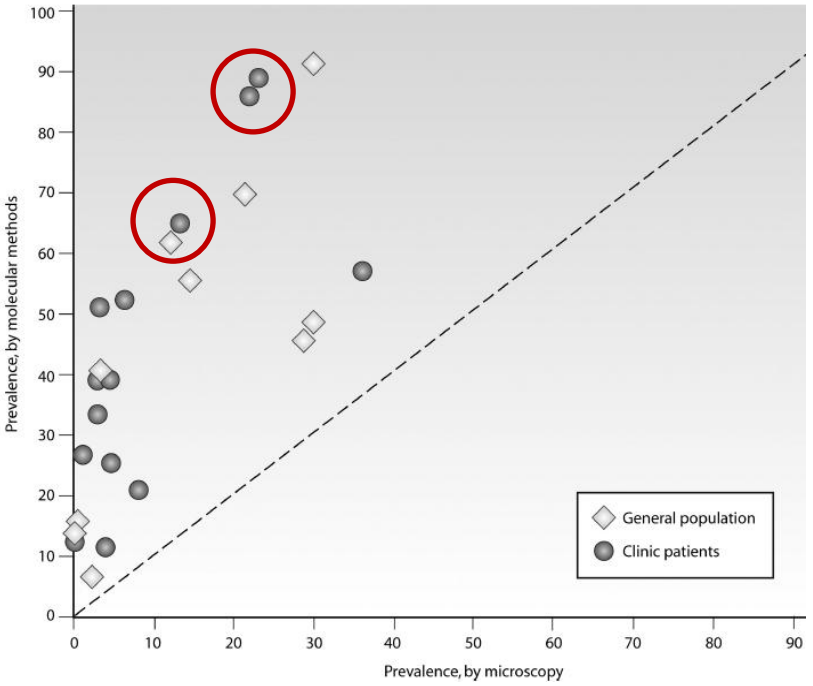
Okell C, Ghani A, Lyons, E et al. JID 2009: 200: 1509-17

Draft Recommendations

- *The majority of infections with asexual parasites have gametocytes detectable by molecular amplification methods. All malaria infections (microscopic and submicroscopic) should be considered as potentially infectious and able to contribute to ongoing transmission. There is no operational need for routine detection of gametocytes in malaria surveys. For research applications, nucleic acid sequence-based amplification (i.e. QT-NASBA or real time qPCR) are the recommended gametocyte detection tools.*

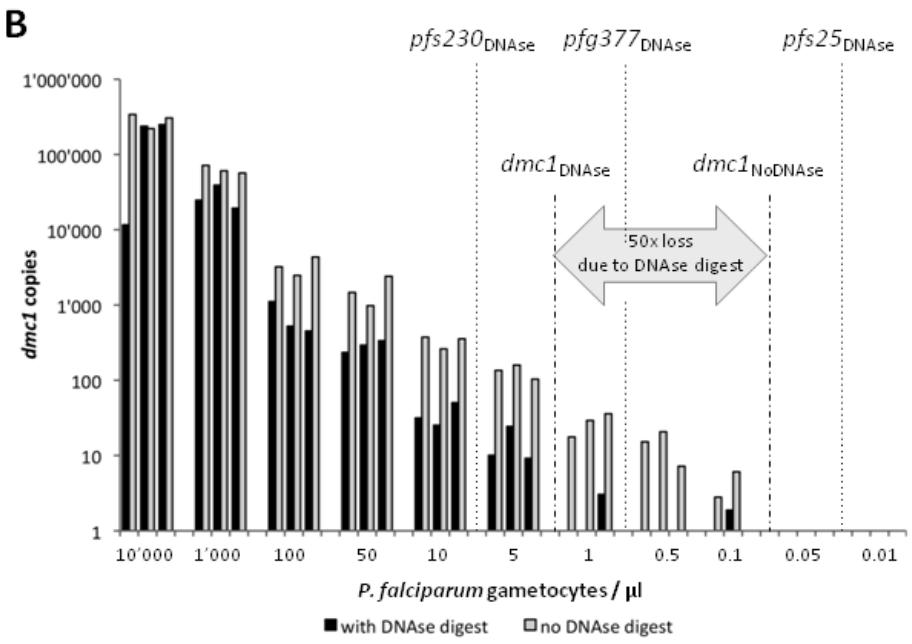
The need to detect gametocytes

- YES:
  - Transmission reducing interventions
  - Assessing the human infectious reservoir
  - Understanding the dynamics of infections
- NO:
  - community surveys for interventions
  - molecular tools have done their work
- **<u>Infection = infectious / soon to be infectious</u>**



Courtesy of: Teun Bousema, Lucy Okell, Andre Lin Ouedraogo, Chris Drakeley

Detecting submicroscopic gametocytes



Light Microscopy:
-very limited sensitivity

qRT-PCR:
-Pfs25 is most abundant target

Sampling strategies:
-RNA protective buffers
-Filter papers stored at -80
-Filter papers stored at -20
-Not in saliva (18S possible)
-Not in urine (18S possible)

Courtesy of: Teun Bousema, Lucy Okell, Andre Lin Ouedraogo, Chris Drakeley

Felger, unpublished data
Wampfler *et al.* PLoS ONE 2013

Jones *et al.* Malar J 2012
Pritsch *et al.* Malar J 2012,
Kast *et al.* Malar J 2013

Draft Recommendations

- *Common standards for nucleic acid based assays should be developed, including use of the WHO International Standard for P. falciparum DNA NAT assays and development of standards for other Plasmodium species, particularly P. vivax should be undertaken. A standard operating procedure should be developed which defines methods for sample collection, extraction, and the recommended equivalent quantity of blood to be added to the assay.*
- *Development of an international, external quality assurance system is strongly recommended to ensure that data obtained from nucleic acid amplification assays are reliable and comparable.*

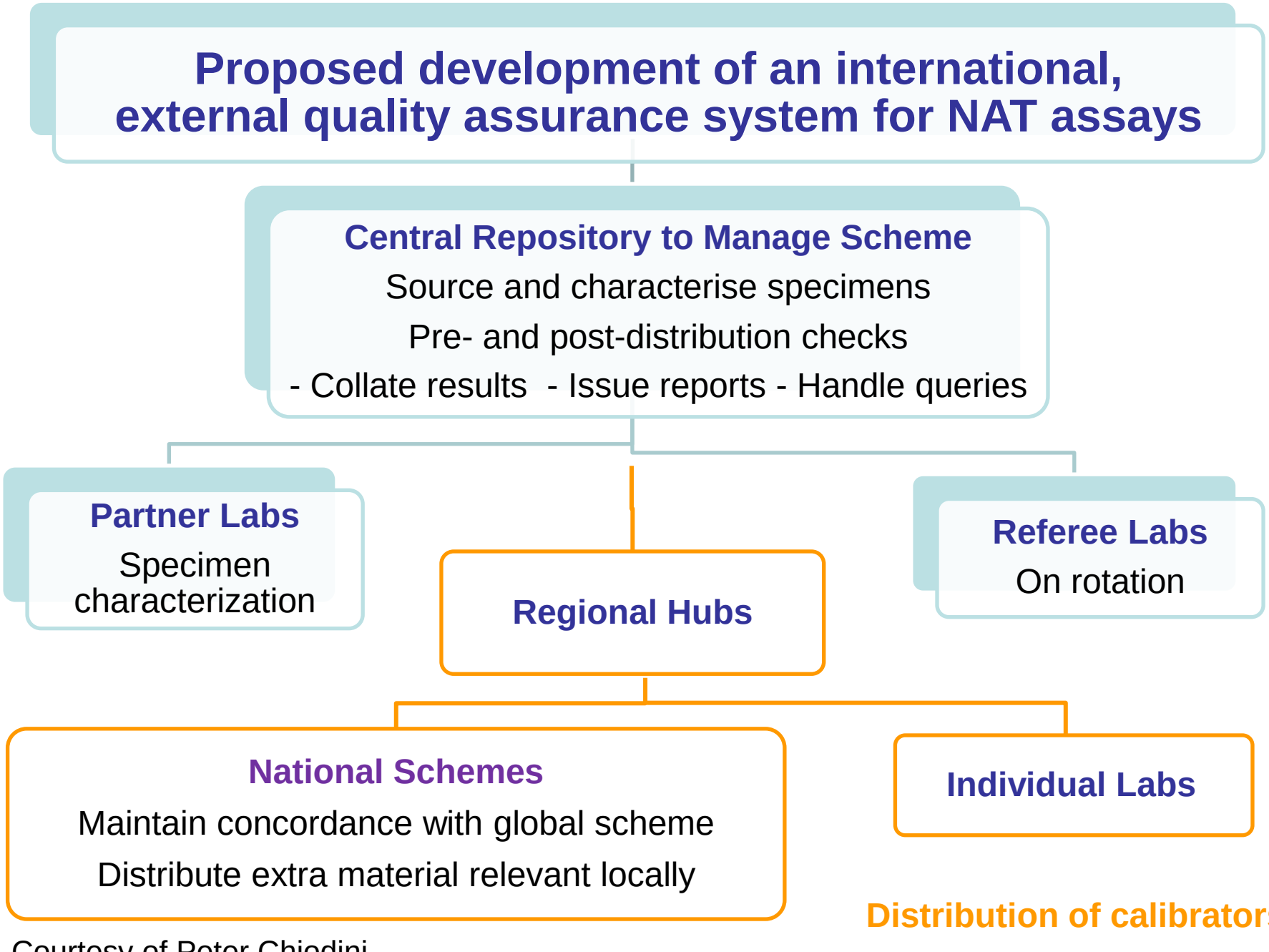
The *M*inimum *I*nformation for Publication of *Q*uantitative Real-Time *E*xperiments: The MIQE Guidelines

Bustin S *et al*. *Clinical Chemistry* 2009, 55:611-622

- *"Lack of consensus on how to perform qPCR experiments"*
  - Possible quantification calibrators:
    - Synthetic RNA or DNA oligonucleotides
    - Plasmid DNA constructs
    - cDNA cloned into plasmids
    - RNA transcribed in vitro
    - Reference RNA pools
    - RNA or DNA from specific biological samples
    - **Internationally recognized biological standards**

- Comparative evaluation of 7 published real-time PCR assays for the detection of malaria following MIQE guidelines
  Alemayehu et al. *Malaria Journal 2013, 12*:277
  - 7 published qPCR assays detecting *Plasmodium* spp or *P.falciparum* compared using standard DNA and samples from a clinical trial: 6/7 assays showed sensitivity lower than what have been published.

Proposed development of an international, external quality assurance system for NAT assays



Courtesy of Peter Chiodini

Draft Recommendations

- *There is a need for standardisation of reagents (antigens and controls), assay methodologies and analysis for malaria serology. Until that becomes available there is a limited role for serological assays in the routine operational monitoring of transmission in elimination settings, but they may still have a role for epide-miological surveys in certain elimination settings.*

Contribution of submicroscopic parasitaemia to malaria transmission

- Uncertainty over contribution of transmission, i.e. estimated submicroscopic parasitaemia source of 20% mosquito infections when malaria slide prevalence is:
  - < 0.5% (Young *et al.*, 1948) *
  - < 4% (Jeffery and Eyles., 1955) *
  - < 24% (ALO, unpublished data) *

Table 1 | Infectiousness of submicroscopic parasite carriers versus slide-positive carriers.

Study	Country	N people	N mosquitoes	% Mosquitoes infected, slide-positives	% Mosquitoes infected, submicroscopic infections
Ouedraogo 2011 (ALO, unpublished data)	Burkina Faso	260	10,244	12.8	3.2
Jeffery and Eyles[29]	USA	95	10,096	20.3	2.0
Coleman *et al.*[30]	Thailand	237	9,210	0.4	0.2*
Young *et al.*[7]	USA	124	6,600	8.4	0.5*

Proportion of mosquitoes infected during human-to-mosquito transmission studies. In two of these studies, the prevalence of submicroscopic carriage among microscopy-negatives was not assessed and therefore this was estimated using the relationship in Fig. 1a.
*Prevalence of submicroscopic carriers estimated.

* *Estimated by Okell C, et al. NC 2012:* 3:1237 DOI: 10.1038/ncomms2241

Draft Recommendations

- *There is a need for more research to understand better the contribution of submicroscopic infections in malaria transmission in low endemic settings and to identify which diagnostic strategies and NAA-based diagnostic techniques are most cost-effective in accelerating malaria elimination, compared to conventional malaria elimination methods. Additionally, markers to identify recent malaria infections, and diagnostic tools that detect P. vivax hypnozoites are needed.*

Available NAA methods for diagnosing low density malaria infections

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.

Applications of malaria diagnostics in low transmission settings (i)

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.

Applications of malaria diagnostics in low transmission settings (ii)

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 minimise 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.

Summary of key points

- Quality assured RDT and microscopy are the primary diagnostic tools for clinical management of malaria in all epidemiological situations, and for routine malaria surveillance in most malaria-endemic settings.
- Submicroscopic *P. falciparum* and *P. vivax* infections are common in low as well as high transmission settings. The majority of infections with asexual parasites have gametocytes detectable by molecular amplification methods, and do contribute to malaria transmission.
- The use of NAA methods by malaria programs should be considered for epidemiological surveys aimed at mapping prevalence of malaria, foci investigations, mass screening and treatment strategies, and screening of special groups (e.g. border screening) when the aim is also to detect submicroscopic infections.
- Molecular tests with analytical sensitivity of 2 parasites/µl should be used for detection of infection in asymptomatic individuals.
- Development of an international, external quality assurance system is strongly recommended to ensure that data obtained from nucleic acid amplification assays are reliable and comparable.

WHO Evidence Review Group on
Malaria Diagnosis in Low Transmission Settings

WHO Headquarters, Geneva, 16-18 December 2013

Meeting Report

In recent years, the application of nucleic acid amplification (NAA)-based diagnostic tools to detect malaria in the context of epidemiological surveys and in research endeavours has increased significantly. Many different assays are available with a superior diagnostic performance to microscopy and rapid diagnostic tests. In order to develop recommendations on the role of molecular diagnostic tests for malaria in low transmission areas and to address operational questions raised by national health authorities, WHO/GMP convened a meeting of experts to review the available evidence.

The ERG proposed the following recommendations for consideration by the Malaria Policy Advisory Committee.

Recommendation 1

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

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

Recommendation 3

Submicroscopic Plasmodium falciparum and P. vivax infections are common in low as well as high transmission settings. A number of nucleic acid amplification techniques are available and are more sensitive in detection of malaria compared to RDTs and microscopy. The use of NAA methods by malaria programs should be considered for surveys aimed at mapping prevalence of malaria, including submicroscopic infections, and to increase the power of surveys at low transmission intensity.

Recommendation 4

The majority of infections with asexual parasites have gametocytes detectable by molecular amplification methods. All malaria infections (microscopic and submicroscopic) should be considered as potentially infectious and able to contribute to ongoing transmission. There is no operational need for routine detection of gametocytes in malaria surveys. For research applications, nucleic acid sequence-

based amplification (i.e. QT-NASBA or real time qPCR) are the recommended gametocyte detection tools.

Recommendation 5

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

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

Recommendation 6

There is a need for standardisation of reagents (antigens and controls), assay methodologies and analysis for malaria serology. Until that becomes available there is a limited role for serological assays in the routine operational monitoring of transmission in elimination settings, but it may still have a role for epidemiological surveys in certain elimination settings.

Recommendation 7

There is a need for more research to understand better the contribution of submicroscopic infections in malaria transmission in low endemic settings and to identify which diagnostic strategies and NAA-based diagnostic techniques are most cost-effective in accelerating malaria elimination, compared to conventional malaria elimination methods. Additionally, markers to identify recent malaria infections, and diagnostic tools that detect P. vivax hypnozoites are needed.

List of abbreviations

CHMI	Controlled human malaria infection trial
EIR	Entomological inoculation rate
FSAT	Focal screening and treatment
LAMP	Loop-mediated isothermal amplification
MDA	Mass drug administration
MIQE	Minimum information for publication of quantitative real-time PCR experiments
MSAT	Mass screening and treatment
NAA	Nucleic acid amplification
NASBA	Nucleic acid sequence-based amplification
PCR	Polymerase chain reaction
PPC	Preferred product characteristics
QA	Quality assurance
qPCR	Quantitative polymerase chain reaction
RDT	Rapid diagnostic test
Ro	Basic reproduction number
Rc	Reproduction number with control measures in place

Background

Light microscopy and antigen-detecting rapid diagnostic tests (RDTs) are the diagnostic tests currently recommended to guide the management of clinical malaria [1]. The use of malaria RDTs has increased in many malaria endemic countries to confirm suspected malaria cases and also for population surveys undertaken to monitor change in malaria trends in high risk populations.

Microscopy and/or RDTs, when used in epidemiological surveys, underestimate the prevalence of low density parasite infections (<100parasites/µl). A systematic review of 42 published malaria prevalence surveys which compared prevalence of *Plasmodium falciparum* based on light microscopy examination of blood slides with polymerase chain reaction (PCR)-based techniques, reported that the prevalence of infection by microscopy was, on average, around half of that measured by PCR [2]. A subsequent review by the same authors showed that sub-microscopic malaria infections are relatively more common in adults than in children and in low rather than in high endemic settings, and that when transmission reaches a very low level, submicroscopic carriers can be the source of 20-50% of all human-to mosquito transmission [3]. However, understanding of the contribution of these low density, submicroscopic infections to disease transmission is based on a limited number of studies.

Due to the limitations of microscopy and RDTs, nucleic acid amplification-based techniques, which are several orders of magnitude more sensitive, are being used increasingly for epidemiological studies, investigations of the origin of infection, analysis of pre-patent parasitaemia in controlled human malaria infection (CHMI) trials, in drug efficacy trials and drug resistance research. They are also being used for the evaluation of new strategies/interventions aimed at transmission reduction, i.e. mass drug administration (MDA), mass screening and treatment (MSAT) and focal screening and treatment (FSAT).

Small subunit 18S ribosomal RNA (18SrRNA) molecular amplification, first exploited by Snounou et al. [4] using a nested PCR technique is the most widely used NAA in malaria diagnostic research, and has been both adopted and adapted by many scientists. Real-time quantitative PCR (qPCR) and nucleic acid sequence-based amplification (QT-NASBA) assays can be used to determine parasite density. More recently, a new commercial molecular assay based on loop-mediated isothermal amplification (LAMP), which uses simpler equipment and is less time-intensive than conventional PCR, has been developed [5]. LAMP can be used for the qualitative detection of *Plasmodium* parasites using a visual or automated read-out and is independent of expensive thermal cyclers. LAMP can differentiate between *P. falciparum* and non-*falciparum* infections, Sensitivity is reported to approach that of nested PCR [6], and LAMP can also be adapted for use on a real-time platform [7].

A lack of clear consensus on standardized methods for qPCR makes it difficult to interpret and compare results obtained by different research groups using this method. In an effort to improve the consistency of real-time PCR (qPCR), specific guidelines were developed in 2009 on Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) [8]. Results of comparative performance of several qPCR assays using these guidelines have been published only recently [9].

WHO does not currently provide guidance to countries regarding the programmatic suitability of molecular diagnostics or guidelines on how to utilize the information that would emerge from their use. Quality assured microscopy is still officially considered the gold standard by WHO, despite large bodies of evidence that shows that PCR and other nucleic acid amplification-based assays are more sensitive than microscopy. There is a need to develop guidance on indications for use, assay selection and quality assurance/control for PCR and other molecular diagnostic techniques for the specific conditions in which use of these malaria diagnostic tools may be appropriate.

1. Objectives

The specific objectives of the consultation were to:

1. Review current knowledge on the contribution of sub-microscopic parasitaemia to transmission, particularly in areas with low transmission.
2. Review the diagnostic performance, technical and resource requirements of available nucleic acid amplification (NAA) methods for diagnosing low-density infections with sexual and asexual malaria parasitesand to recommend the most suitable methods of diagnosis for use in population surveys and active case investigations.
3. Review requirements to ensure quality for NAA methods and build capacity to support programmatic interventions in pre-elimination and elimination settings.
4. Review and suggest revisions to current WHO recommendations for malaria diagnostic approaches in low transmission settings.
5. Review the malaria diagnostic R&D pipeline and reach consensus on preferred product characteristics of new diagnostic tools to meet public health needs for malaria elimination.

2. Process

A series of presentations were made during the first two days of the meeting under five themes:

1. Malaria epidemiology in low transmission settings.
2. Current molecular diagnostic techniques for malaria.
3. Quality assurance of molecular diagnostic technologies for malaria.
4. Field applications of molecular diagnostic technologies and serology for malaria.
5. Future malaria diagnostic technologies for low transmission settings.

When possible, presenters were requested to present the evidence as a systematic review, highlighting any growing consensus and remaining evidence gaps. Finally, the experts were divided into three working groups to discuss specific questions around three themes:

- Malaria epidemiology in low transmission settings.
- Current molecular diagnostic techniques for malaria.
- Field applications of molecular diagnostics for malaria.

Descriptions of transmission intensity settings are included in the Table below. Meeting discussions and recommendations were made in the context of settings where there is already widespread availability of diagnosis (by RDT and/or microscopy) and treatment and where malaria endemicity is low (e.g. prevalence < 10%).

Table 1 Malaria surveillance in different transmission settings and phases of control			
	Control phase		**Elimination phase**
Transmission:	**High & moderate**	**Low**	**Very low**
Parasite prevalence (2-9 yrs):	>10%	<10%	
Incidence:	Cases and deaths common and concentrated in <5yrs	Cases and deaths less common distributed according to mosquito bite exposure	Cases sporadic
	Limited temporal variation	Variable within and between years Risk of epidemics	Imported cases may be high proportion of total
	Limited geographical variation	Geographical heterogeneity Concentrated in marginal populations	Focal distribution
Fevers:	Proportion of fevers due to malaria relatively large	Proportion of fevers due to malaria small	Proportion of fevers due to malaria very small (though may be high in certain foci)
Health facility attendance:	High proportion due to malaria	Low proportion due to malaria	
Vectors:	Efficient	Controlled efficient/ inefficient	Controlled efficient/ inefficient
Aims of programme:	Mortality & case reduction	Case reduction	Eliminate transmission
Surveillance system			
Resources:	Low expenditures per head Low quality and poor accesibility of services	Widespread availability of diagnostics and treatment	Resources to investigate each case
Data recording:	Aggregate numbers	Aggregate numbers Lists of inpatients and deaths → lists of all cases	Case details
Investigation:	Inpatient cases	Inpatient cases → all cases	Individual cases

Source: WHO (2012). Disease surveillance for malaria elimination: an operational manual

3. Evidence reviewed

3.1 Malaria epidemiology in low transmission settings

At all levels of *Plasmodium* transmission, there are individuals with submicroscopic infection present in the population. The relative proportion of submicroscopic and microscopic infections varies between settings, depending on factors such as age, transmission intensity and immunity. In low transmission settings, submicroscopic infections may represent a significant fraction of infections, but the major determinants of the contribution of submicroscopic infections to malaria transmission are not clear. Submicroscopic infections are prevalent both in "stable" low endemic areas and in those areas experiencing recent reductions in transmission [2].

While a systematic review indicated a relationship between microscopy and PCR prevalence [2], the proportion of all infections in a population which are submicroscopic is not predictable in any given situation, particularly in areas of low transmission. Thus, when quantification of submicroscopic infections is needed, this has to be measured directly.

The duration of submicroscopic infection is variable, but often lasts for several months. In areas of seasonal transmission, submicroscopic infections can persist during the entire season of very low transmission [10]. There is evidence that, in areas with high seasonality and in the absence of treatment, an individual with a sub-microscopic infection before the low transmission season can be infectious to mosquitoes during the next high transmission season. Gametocytes are grossly underestimated by microscopy in both high and low transmission settings [11], but, among patients with clinical malaria gametocytes are usually present at presentation across the full range of transmission settings in Africa [12-15].

Molecular methods show that a large proportion of asymptomatic individuals, including adults, have sub-microscopic gametocytaemias (Ouedraogo et al., unpublished). Membrane-feeding experiments show that a significant fraction of individuals with submicroscopic infection are infectious to mosquitoes throughout the transmission season, and that this fraction decreases with age (Ouedraogo et al., unpublished). There are limited data to describe the dynamics of submicroscopic infections, for example their contribution to transmission prior to the onset of clinical symptoms arise and whether submicroscopic infections are always preceded by symptomatic and high density stages of infection.

The likelihood of transmission depends not only on the density of gametocytes and their infectivity to malaria vectors but also on the many determinants of the efficiency of the vector (vectorial capacity). The specific threshold of gametocyte prevalence below which no transmission is possible is not known, but likely dependent on the interaction between the vectorial capacity and the proportion of gametocyte carriers in the population. Geographical heterogeneity in transmission exists in low transmission settings, and this contributes to spatial heterogeneity in host immunity [16].

The use of nucleic acid-based tests with higher sensitivity and less systematic bias compared to microscopy and RDTs, allows for an increase in the power of surveys to detect hotspots of transmission in low transmission settings.

P. vivax blood-stage infections tend to be lower density than *P. falciparum* infections but gametocytes can be found very early in *P. vivax* infection. Immunity appears to be acquired more quickly against *P. vivax* than against *P. falciparum* in high and low transmission settings. A survey on the Thailand-Myanmar border, indicated that the majority of *P. vivax* infections are asymptomatic, 81% were low density (<100 parasites/µl) and up to 80% of subjects had gametocytes (Nuitragool et al., unpublished). In community cross-sectional surveys where *P. vivax* prevalence is low by microscopy, a high proportion of all infections are submicroscopic. On the contrary, it seems that, among febrile patients, fewer submicroscopic *P. vivax* infections are found. Factors which may contribute to *P. vivax* submicroscopic infections include i) parasite factors such as growth characteristics and virulence, population homogeneity and relatedness of circulating strains; ii) innate resistance among hosts due to Duffy antigen, haemoglobin variants and G6PD deficiency; iii) acquired resistance as a result of repeated infections, duration of infection or heterogeneity of the parasite population.

There is some indication that very low-density *P. vivax* infections do contribute to ongoing transmission but data are very limited [17, 18]. It is also apparent that RDT and microscopy do not detect the full prevalence of *P. vivax* infection because of the large proportion of submicroscopic infection in low transmission settings. There is currently no diagnostic tool able to directly detect *P. vivax* hypnozoites.

In Temotu Province, Solomon Islands, a large-scale survey found a high proportion of all *P. falciparum* and *P. vivax* infections to be submicroscopic and not associated with age. In this area, submicroscopic *P. falciparum* infections were associated with low genetic diversity, with imported strains causing clinical disease. However *P. vivax* infections showed high genetic diversity, and the prevalence of

submicroscopic infections could be due to multiple factors, such as immunity, infection duration or other host factors [19].

Key Conclusions:

- Submicroscopic *P. falciparum* and *P. vivax* infections are common in low transmission settings and contribute to transmission.
- Outside research studies, there is no requirement to specifically detect gametocytes since a very high proportion of individuals with blood stage infection can be shown to harbour infectious submicroscopic gametocytes when molecular diagnostic tools are used. All infected individuals should be considered as potentially infectious and thus potentially able to contribute to transmission.

3.2 Current molecular diagnostic techniques for malaria

A number of different PCR diagnostic techniques exist: single step, nested, multiplex and quantitative. Alternative nucleic acid amplification techniques have been developed which do not need thermocyclers, the most common are loop-mediated isothermal amplification (LAMP) and nucleic acid sequence-based amplification (NASBA).

The nucleic acid target selected influences PCR results with more than 65 primer sets and >5 targets widely in use [20]. 18S is the most commonly used target, having a moderate copy number and being well conserved, but it performs less well for *P. malariae* and *P. ovale* than for *P. falciparum*. Alternative target genes are being explored which have a higher copy number and this may have implications for reducing limits of detection. Detection of total nucleic acid can significantly increase sensitivity [21], but has increased cost due to the need for a reverse transcriptase step.

Molecular diagnostic techniques generally have significantly higher sensitivity than the best microscopy. On average, a good microscopist can identify 50 parasites per µl blood (p/µl) while the expert microscopist will struggle to detect regularly infections <20 p/µl (Chiodini, unpublished). The limit of detection for RDTs is generally in the order of 100 p/µl, while lab-based PCR methods generally have published limits of detection of <5 p/µl [22, 23].

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.

The quantity of blood used for amplification and extraction efficiency are the crucial factors when comparing limits of detection between methodologies in very low transmission settings where low density infections are likely. It was recommended that at least 50µl blood be collected from individuals[3] when molecular testing is to take place, and that a minimum of 5µl blood should be used in the assay. To be a "significant improvement" over expert microscopy, molecular (and other non-molecular) methods must be able to detect 2 parasites/µl (10 parasites in 5µl blood analysed) or fewer.

Some amplification methods employ chemistries that allow DNA replication without the need for denaturing and annealing steps and can thus operate at a single temperature, obviating the need for a thermocycler. Isothermal amplification methods are therefore potentially of use in basic laboratories or at field level. LAMP is highly specific (it uses 4-6 primers), fast (15-60 minutes), and the result is assessed by visual detection of fluorescence or turbidity [41]. A closed system of dry-down reaction tubes, stable up to 30˚C, is available for LAMP in a field setting (Loopamp™). Methods to allow processing of high numbers of samples for LAMP over a relatively short period of time need further improvement. For basic laboratories, boil and spin is the simplest and lowest cost extraction method. FIND is developing *P. vivax* probes (currently Loopamp™ Pan or Pf probes are available). There are some indications that a sealed system LAMP may require less training time for laboratory staff than that needed for high quality microscopy services.

High volume quantitative PCR improves the limit of detection by increasing the quantity of target DNA in an assay, improving sensitivity by up to 50 times (Imwong et al., unpublished). The best target genes for this type of PCR are Pan species or *P. falciparum* 18S RNA and a standard control using the *P. falciparum* clone 3D7. A sequential process is suggested, first conducting Pan qPCR and then moving to species-specific targets. This assay has a good amplification efficiency, and showed high diagnostic specificity and low variation across assays. Development of the assay is ongoing to improve its sensitivity in species detection. This assay has a higher cost than LAMP, but the additional sensitivity offered by high volume qPCR may be justified in specific settings, such as part of artemisinin resistance containment efforts.

Cost effectiveness analyses are required to inform decisions regarding selection of the best diagnostic method to use for different operational purposes. For example, in some circumstances, it may be more cost effective to test a larger number of individuals with a less sensitive test than testing a small number of individuals with a highly sensitive test.

More research is needed to assist control programmes in low transmission settings to decide whether to prioritise high throughput point-of-care strategies which have higher limits of detection, or if the lowest possible limit of detection is more important than having a high throughput test. The location of sample testing also influences this decision, with the most highly sensitive available at high cost at central reference laboratories, compared to PCR machines and protocols which may be appropriate for lower level health facilities but with slightly reduced detection performance but improved portability and ease of use.

[3] 50µl is an achievable volume to collect via fingerprick; the template can be sufficiently concentrated to allow an aliquot equivalent to 5 µl of blood to be used, and this volume is sufficient to repeat the assay should confirmation be needed

4.2.1 Applications of malaria diagnostics tests in low transmission settings

In the absence of evidence on cost effectiveness and public health impact of transmission reduction interventions using NAA tests, some general guidance is provided to inform the selection of NAA based assays for use in low transmission settings.

There was consensus that in all settings NAA-based assays should meet the following characteristics:

1) The tests should detect genus and have the capacity for post-processing species differentiation, if regionally relevant.
2) Quantification is not essential, but may be appropriate in some contexts. Qualitative scoring is likely to be sufficient for most settings.
3) The limit of detection of each assay should be established against the WHO International DNA standard panel (for *P. falciparum*) using standardised methods.
4) Gametocyte detection is not essential but may be needed for research purposes.

In addition, it was agreed on the following applications:

1) *Routine surveillance and passive case detection in low transmission settings*
   a) Based on appropriate case definition of suspected malaria, microscopy and RDTs are sufficient.

2) *Malaria epidemiological surveys in low transmission settings*
   a) A substantial proportion of infections are missed by microscopy and RDTs due to the presence of low parasite density infections.
   b) A molecular test (or other technology) with analytical sensitivity of ~2 parasites/µl should be used to provide a significant improvement over expert microscopy. Classic PCR, qPCR and LAMP meet this specification if performed properly, but other validated,
   c) non-molecular tests with similar performance would also be potentially acceptable options.
   d) It is recommended to collect at least 50µl blood from each individual, and to ensure that the eluate used in the assay is derived from a minimum of 5µl blood. It may be acceptable to use smaller quantities of blood for assays with RNA targets if these targets are homogeneous mixed in the extracted material.
   e) Rapid turnaround times are not a priority.
   f) Internal and external quality assurance procedures should be in place.

3) *Foci investigations: reactive infection detection following identification of an index case*
   a) A molecular test (or other technology) should be used which has an analytical sensitivity of 2 parasites/µl or 10 parasites in 5µl blood analysed.
   b) Turn-around time should be <48 hours to allow prompt follow up and treatment of positive individuals.
   c) A context-dependent decision should be made regarding the balance between providing high throughput services of high sensitivity but at a location far from the field, and providing lower throughput and lower sensitivity molecular testing close to the point of care with fast results.
   d) Field-adapted classic PCR, qPCR and LAMP methods are appropriate, and mobile laboratories may be a useful option.
   e) QA including EQA should be in place for the analysis technique chosen.

4) *Mass screening and treatment*
   a) RDT and microscopy are not sufficiently sensitive for mass screening and treatment programmes in low endemicity settings
   b) A moderate throughput test with analytical sensitivity of 2 parasites/µl should be used to allow identification of asymptomatic and low-density infections.
   c) Results should ideally be available on the same day as testing to maximise follow up of individuals and provision of treatment
   d) Field-adapted classic PCR, qPCR and LAMP methods are appropriate, and mobile laboratories may be a useful option.
   e) QA including EQA should be in place for the analysis technique chosen.

5) *Screening of special populations (e.g. at border crossings)*
   a) The local context will determine which are the most appropriate and cost-effective tools to use, and whether screening at borders is a feasible and useful approach.
   b) If screening of special populations is deemed appropriate:
      i) RDT or microscopy should be used for symptomatic infections only.
      ii) Molecular tests with analytical sensitivity of 2 parasites/µl should be used for detection of infection in asymptomatic individuals.
      iii) Results should be provided on the same day to minimise loss to follow-up.

6) *Additional characteristics to be considered*
   a) Common standard operating procedures should be used for the methods deployed, with positive and negative controls in use and all assays should be conducted under conditions of good laboratory practice.
   b) An objective reading (i.e. clear, unambiguous thresholds for positive and negative results that are independent of reader bias) of the PCR/LAMP endpoint result may be beneficial.
   c) Training programmes should be developed, potentially through the regional hubs responsible for coordinating the EQA system.
   d) Standards should be developed for *P. vivax, P. ovale*, *P. malariae*, *P. knowlesi* species in addition to *P. falciparum*.
   e) If RNA assays are to be used, laboratories should develop and adhere to RNA standards.
   f) Negative controls should be used in all molecular assays.
   g) The method of blood collection should be decided by local context. While blood spots on filter paper are simple to collect in the field, extraction from filter papers is laborious, and the volume of blood available relatively small. New products are becoming available which include DNA/RNA preservatives and can be used for collection of more than 50µl blood, as well as storage and transport of samples.

Key Conclusions

- Nucleic acid amplification techniques are more sensitive and specific than RDTs and microscopy in the identification of low density infections but until more evidence is available, their use should generally be restricted to low or very low transmission setting and only considered where it is operationally useful to identify asymptomatic and low density infections.
- 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 (10 parasites in 5µl blood analysed or fewer).
- Isothermal amplification methods such as LAMP are potential useful in peripheral laboratory settings, offering a more sensitive test than RDT or microscopy. Development of a commercialized closed system of dry-down tubes that are stable up to 30˚C, as well as reduced training time to achieve proficiency in LAMP compared to high quality microscopy, support the likely utility of LAMP at a field level.
- It is recommended that at least 50µl blood be collected from individuals when molecular testing is to take place, and that the eluate used in the assay is derived from a minimum of 5µl blood .
- Molecular tools may have little additional benefit in stratifying risk of malaria at macro-epidemiological scales, but may have additional value at micro-epidemiological scale and in areas of very low transmission.
- High volume qPCR is a technique which has demonstrated improved limits of detection as a result of increasing the quantity of blood used for the qPCR assay. While costing more than other NAA methods, it may be of use in specific contexts where test with very high sensitivity are required.

3.3 Quality assurance of molecular diagnostic technologies for malaria

The need for a WHO International external quality assurance (EQA) scheme was discussed at the meeting and there was full consensus that is an essential requirement and that it should be developed before any large-scale adoption of NAA based methods by control programmes. The scheme should ideally be managed by a central repository responsible for sourcing and characterising specimens, conducting pre- and post-distribution checks on specimens, collating results, issuing reports and handling queries. In addition to the central repository, partner laboratories would support specimen characterisation, and reference laboratory designation would be rotated. The central repository would distribute samples to regional hubs. Regional hubs are expected to set guidelines and develop training manuals. The regional hubs would also lead training, either hands-on training or by distribution of training kits. Regional laboratories would also be responsible for assessment of individual laboratory performance including species identification, and providing a dilution series to determine limit of detection, as well as challenge samples. Scoring schemes are necessary with progressive reinforcement of the message for laboratories that continue to perform poorly. Poor performance would prompt offers of support and retraining. Standard materials for EQA of DNA-based methods are currently only available for *P. falciparum* [42], but there are efforts ongoing to produce genus-specific markers. Alternatively, accrual and pooling of samples may allow production of standardised material. Standard materials to support various assays might include wild-type parasites; whole blood; leucodepleted blood; parasites cultured in human blood; hybrid calibrator seeded into whole blood; DNA seeded into whole blood. In turn these standards could be made available in a variety of formats including frozen

blood, freeze dried blood, dried blood spots etc. Markers for artemisinin resistance, once fully validated, could also be included in the international EQA system.

In addition to an international EQA scheme, some countries may wish to establish a national EQA scheme. These should maintain concordance with the WHO scheme, and be in a position to distribute additional locally-relevant materials to laboratories within the country. Internal quality assurance should also take place within each laboratory, including standardised controls and generation of control charts to track control data over time. The initial focus for participation in this molecular methods EQA system would be regional laboratories in elimination settings.

- Key Conclusions:

- Common standard operating procedures and standards for nucleic acid based assays should be developed, including use of the WHO International Standard for *P. falciparum* DNA NAT assays and development of standards for other Plasmodium species, particularly *P. vivax*.
- Development of an international, external quality assurance system is strongly recommended to ensure that data obtained from nucleic acid amplification assays are reliable and comparable. These should be in place before any large-scale adoption of NAA based methods by control programmes.

4.3.1 Quantitative PCR: challenges of harmonisation

There is a lack of consensus on how best to perform and interpret qPCR experiments, and challenges in replicating published results, in particular those related to sensitivity and specificity [9]. Guidelines have been published on the minimum information for publication of quantitative real-time PCR experiments (MIQE) to ensure integrity of the scientific literature, promote consistency between laboratories and increase experimental transparency. There is no need to recommend that all laboratories perform the same assay, so long as the assay meets MIQE and performance characteristics, which includes the use of a consistent reference standard [8]. A test with a known limit of detection should be used in epidemiological studies to allow comparability of findings with other data.

Requirements for harmonisation of qPCR methods for controlled human malaria infection trials include:
1) The assay developed should follow the MIQE rules.
2) The algorithm used to determine positive results should be standardised for CHMI and field studies; since the repeatability of assay results is influenced by Poisson distribution of parasites at the limits of detection, not every replicate will generate a positive result.
3) The assay should not be platform-specific, allowing the assay to be transferred from one platform to another, with performance re-evaluated on each new platform.
4) Controls should be in place to account for extraction and inhibition, assay performance, contamination and false positives.

3.4 Field applications of molecular diagnostic technologies for malaria

When approaching elimination, it is likely that it will be more important to identify the last community with Rc>1 where ongoing transmission is possible, rather than identifying the “last parasite standing”. It is not clear at present which interventions can be promoted to accelerate malaria elimination, and

research on the use of focal screening and treatment (FSAT) or mass drug administration strategies (MDA) is ongoing. A major threat to elimination would be from reintroduction or expansion of transmission into malaria receptive foci with an Rc>1.

Data from South America following down-scaling of indoor residual spraying (IRS) indicate that re-introduction of cases is possible [43]. In areas where the Rc>1, deployment of FSAT may have the potential to reduce transmission but is highly unlikely to lead to interruption of transmission. Research is ongoing on additional requirements to increase the impact of FSAT in relation to the seasonal pattern of malaria transmission, knowledge of vector bionomics and ecology and understanding of human movements. Health services and malaria control programmes considering FSAT should analyse the cost-benefits of different strategies in target areas, the expected gains from accelerating reduction in transmission, and the probability of malaria reintroduction from outside areas. The fundamentals of health systems should not be forgotten in the drive to elimination, including good case management practices, effective vector control, supply chain management, information systems and data reporting, as well as training of staff.

For passive case detection, the role of diagnostic tools is similar for both control and elimination, mainly for testing febrile patients suspected of having malaria, unless there is an obvious alternative cause of fever. Active case detection occurs at the community and household level in high risk locations and population groups, but the term also refers to repeated surveillance activities, border screening and mass- or focussed-screen and treat programs. However, the extent to which it is necessary to identify all infections, particularly if Ro<1 in the location and therefore transmission is likely to be self-limited and end naturally, is currently unclear.

A series of country experiences were presented and discussed, related to the field application of malaria molecular diagnostic tests in low transmission settings, including a review of the role of malaria serology.

The Ministry of Health of Swaziland is targeting malaria elimination for 2015, with only 379 cases reported during a 12-month period from 2012-2013; 26% of these cases was attributed to local transmission. The Malaria Indicator Survey in 2010 included pooled PCR and serological analysis by enzyme-linked immunosorbent assay (ELISA). Very few PCR positive individuals were found during the MIS, and the malaria programme decided that a more targeted surveillance approach was required. Swaziland adopted a system of active case investigation and reactive infection detection by screening residents living within 1km of confirmed infections identified at a health facility. Screening using RDT found very few additional asymptomatic infections. Screening individuals with LAMP resulted in a three-fold increase in detection. Reactive screening was a labour intensive strategy and yielded few additional infections using RDT, but detection of infections was increased by using LAMP. The national malaria control programme is considering using other means to identify hotspots of transmission such as serological data from cross-sectional surveys, or geo-location of confirmed cases who present at a health facility and screening of residents living within 500m (instead of 1km) of a confirmed case.

Zanzibar has experienced a significant reduction in malaria cases over recent years, with transmission now very seasonal and focal. Weekly reporting of malaria cases from health facilities is conducted by mobile phone, with a high reporting rate. An active infection detection approach has been used since 2012, intended to identify and treat asymptomatic infections thereby reducing the parasite reservoir and reducing transmission. Large-scale surveys have also been used to screen the population with various diagnostic tools. Pro-active infection detection in Zanzibar by screening of households of confirmed malaria cases with an RDT failed to detect many additional cases and did not reduce seasonal transmission. Active case detection using PCR did identify additional low density infections not

found by RDT, but samples were analysed at remote laboratories resulting in a long time delay. A pilot study using a commercial LAMP kit for screening gave promising results, finding additional cases missed by RDT without requiring a long processing period and waiting time for the results. The diagnostic tool used for screening should be able to identify both *P. falciparum* and non-falciparum infections, as a large proportion of infections identified by PCR were non-falciparum infections. Cost benefits of the different tools should be considered alongside priorities of the national malaria control programme. RDTs are likely to remain as a point-of-care diagnostic tool, but missed up to 90% of infections at the population level in Zanzibar. LAMP could, in principle, be used as a point of care test but it is more expensive.

Mobile laboratories are an alternative approach to enable PCR tests to be done at field level. In Cambodia a mobile laboratory has been designed in a transportable container with all required equipment and with the capacity to run either from the main power supply or from its own internal generator. In this facility, a trained team of staff were able to analyse up to 350 samples per day, with results-based treatment provided within 48 hours of sample collection (24 hours for the assay, with allowance for repeats due to quality control failures). The mobile laboratory could consistently detect a positive control dilution at 2 parasites per µl. The assay performed best when using 5µl blood, restricting the true sensitivity and limit of detection. While a mobile laboratory is expensive and may pose very complex operational challenges for transport, it may be appropriate for specific remote settings where transport is feasible and faster results are required than would be achieved by sending blood samples to a central laboratory at a long way away.

Key Conclusions:

- For clinical management of febrile patients routine surveillance and passive detection in low endemic areas, the use of microscopy and RDT is sufficient and molecular diagnosis is not currently required.
- Screening and treatment strategies aimed at reducing and interrupting transmission, such as MSAT and FSAT, should be based on molecular tools in order to identify submicroscopic infections as well as higher density infections.
- In settings with low transmission considering malaria elimination, Malaria Indicator Surveys and other similar M&E activities should include a component based on molecular tools, particularly exploring the burden of submicroscopic infection.
- Molecular diagnostics have limited advantages for use in clinical management of travellers from non-endemic countries with suspected malaria.
- Molecular diagnostics can identify submicroscopic placental malaria infections, however it is unclear if these submicroscopic infections in pregnancy are associated with low birth weight or other adverse pregnancy outcomes.

3.5 Field applications of malaria serology

Sero-epidemiology is the measurement of malaria specific antibody responses and is concerned with measuring exposure to infection, not necessarily protection. Antibodies are species and antigen-specific, acquired at infection and lost over time. Age-specific seroprevalence can be used to model sero-conversion rates and correlates with indicators of transmission such as the entomological inoculation rate (EIR), parasite rate and the basic reproduction rate (R_0). In settings with measured parasite rates of zero, estimated sero-conversion rates showed transmission equivalent to an EIR of 2 infectious bites per man per year (Drakeley & Griffin, unpublished). Age-specific seroprevalence rates

have been used to assess changes in malaria transmission over time in Cambodia (Cox & Meek, unpublished) and in The Gambia, changes in exposure associated with behaviour and seasonal changes in exposure in Indonesia [44]. Serological data also are useful in spatial analysis of transmission and identification of hotspots at various scales, from national level to district and village level. A major limitation to the use of serology is the lack of standardised reagents (antigens and controls), variations in the assay methodologies used and in the method of analysis. Defining seropositivity is a key challenge, particularly without the use of standardised controls. Work is ongoing in screening and characterising antigens which represent markers of recent versus historical exposure, including micro-arrays which allow statistical prediction of serological incidence [45] (Bretscher et al., unpublished). While malaria serology has value in characterising malaria exposure in low transmission settings, for its broader use, there is a need for standardisation of methods and more research to identify the lowest acceptable limit of seroprevalence for certification of malaria elimination. Serological indicators are not currently appropriate for single measurements (with the exception of antibody detection in children in an area believed to have no malaria transmission, where they may indicate ongoing transmission), and are most useful to monitor changes in malaria transmission over time.

Key Conclusions:

- Sero-epidemiological methods are useful in assessing changes in malaria transmission over time, as well as identifying behavioural changes in exposure to malaria. Sero-epidemiology can facilitate identification of hotspots and hotpops of malaria transmission, to support evidence-based targeting of interventions. Standardisation of methods and quality assurance are also required for serological analysis methods to optimise their usefulness.

3.6 Applications of molecular diagnostic technologies for travellers and malaria in pregnancy

Molecular diagnostics have limited advantages for use in travellers from non-endemic countries. PCR testing of returning travellers on day 0 would only identify 1 missed infection for every 500-1000 tested [46, 47]. PCR would have limited additional benefit in identifying malaria species in travellers, resulting in clinical impact for 2% of patients [48]In addition, PCR has limited benefit in travel clinics due to increased cost and waiting time for the results, but it may have value in quality control of RDT and microscopy, confirming species and diagnosing recurrent or prolonged fevers of unknown origin when RDT and microscopy are negative and malaria is still suspected.

In low to moderate transmission settings, a review of seven studies found that no hospitalisation or death was identified due to missed malaria infection in pregnant women while using an RDT for clinical case management rather than PCR [49]. RDTs on peripheral blood has been shown to detect 57% and 93% of submicroscopic placental infections in two studies, but there is no conclusive evidence that submicroscopic placental infections are associated with low birth weight [49]. RDTs are likely sufficient to identify women with the highest placental parasite densities who are at highest risk of delivery of a low birth weight baby, and in the future, screening and treatment with RDTs may have a potential to replace intermittent preventive treatment in pregnancy in low endemic areas, or in areas with very high resistance to sulfadoxine-pyrimethamine.

Key Conclusions:

- Currently available evidence indicates that molecular diagnostics have limited advantages for use in clinical management of travellers from non-endemic countries with suspected malaria.
- Molecular diagnostics can identify submicroscopic placental malaria infections. However it is unclear if these submicroscopic infections in pregnancy are associated with low birth weight or other adverse pregnancy outcomes.

4. Future diagnostic technologies for malaria

Trials of non-invasive antigen detection from urine samples are ongoing. Indications are that these tests are functional but have lower levels of sensitivity than tests on blood. New, novel targets for diagnostic assays include glutamate rich protein (GLURP), dihydrofolate reductase (DHFR) and heme detoxification protein (HDP), and these antigens are beginning to be adopted by industry for assay development. Some research is being conducted into thermostable antibodies, and in improving sensitivity by use of fluorescent signals. Various electronic readers have been developed which give a quantitative read out from lateral flow kits, but there have been few formal comparisons of the use of these machines compared to standard technologies. A nano-level approach which is able to detect a single protein molecule in blood may have benefit for high-throughput approaches to screen populations where other molecular methods are not being used [50]. Another new approach involves undertaking PCR directly from blood using gel pads which rehydrate in the presence of a 1:10 dilution of whole blood; these tests appear to have good sensitivity [51-53].

Most of the new technologies for malaria diagnostics in an elimination setting are at a discovery and development stage, although some technologies are already being commercialised. PATH's Project DIAMETER (Diagnostics for Malaria Elimination Toward Eradication: more information available at www.path.org) is working to build consensus on the technical specifications for elimination diagnostics, then support rapid access to the most cost-effective and temporally effective tools in the pipeline. This project is currently at the stage of sharing and reviewing preferred product characteristics (PPC) and developing a taxonomy to clarify terminology between different potential applications related to malaria elimination. Scenarios including point-of-care detection and management, high throughput laboratory infection detection, and methods for pro-active point of contact infection detection will all feed into the PPC.

The following preferred product characteristics for new technologies were discussed at the meeting:

- An ability to detect parasitaemia of ≤2 parasites/µl.
- Need for a sample volume of not more than 50µl blood.
- An assay that is not instrument specific.
- Flexibility in power supply.
- An ability to detect malaria parasites at genus level and then conduct species differentiation on positive samples.
- Results should ideally be available within 16 hours (same working day or early on the following day for patients providing samples just before closing hours), with a maximum waiting time of 24 hours for results.
- The assay should allow processing of 48 samples/person/platform/day.
- Reagents should be stable at 4°C for a minimum of one year, and at room temperature for a minimum of six months.

- Training for conduct of the assay should require no more than five working days, with a minimum number of steps involved in conducting the assay
- Shipping conditions should not require a temperature of less than 4°C
- No hazardous waste should be produced during the assay and there should be no risk of harm for the user.
- The contamination risk is low.
- The assay is affordable.
- The equipment needed to undertake the assay is portable.
- The assay is automated with an objective end-point reading.
- Results are simple to interpret.
- Desirable network connectivity for data transfer.
- The assay specificity for detection of the *Plasmodium* genus is 95% or higher.

5. Ongoing research questions

There is a need for better understanding of the determinants of malaria transmission in low transmission settings in order to develop appropriate strategies to accelerate malaria elimination. In particular the following research questions need to be addressed:

- What frequency of submicroscopic infections become symptomatic as part of the natural history of infection?
- What is the relation between gametocyte density and infectiousness, and what are the main determinant factors influencing this relationship in the carrier population?
- What are the thresholds of gametocyte carriage rate, density and infectiousness at the population level, in relation to the vectorial capacity, below which transmission cannot be maintained?
- In the natural history of infection what is the duration of infectiousness in relation to the duration of infection?
- What are the major determinants of the duration of infectiousness, e.g. strains or super-infection, relapses from liver, or other?
- As transmission falls to a level which may make elimination possible, what are the relative proportions of microscopic and submicroscopic infections that contribute to transmission, and the age-specific changes in the distribution of submicroscopic infections?
- How is the relation between microscopic and submicroscopic infections affected by the persistence of low transmission and the level of malaria immunity in the population?

The participants also underscored the need for a multi-disciplinary research to address the following fundamental question:

- Which diagnostic approaches and molecular diagnostic techniques are cost-effective in accelerating malaria elimination in areas of low transmission, and varying transmission potential (Ro, Rc) , compared to conventional malaria elimination methods?

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Annex 1: Operational questions from countries and responses from the ERG

1. *What are the recommended tests to detect asymptomatic infections in population surveys, active case detection, screening, and case management in elimination settings?*

   The recommended diagnostic test to identify infections for case management remains microscopy or a rapid diagnostic test. For detection of asymptomatic infections in population surveys, active case detection and screening, microscopy and/or nucleic acid-based tests can be used.

2. *What is the gold standard of malaria diagnosis in elimination setting?*

   The current scientific evidence demonstrates that nucleic acid-based tests have the highest sensitivity and sensitivity, but these methods should not be used until methods have been WHO-standardised and quality assurance systems are in place. In the meantime, quality assured microscopy remains a practical gold standard.

3. *What are the recommended diagnostic tools to be used at community level in areas targeted for malaria elimination, considering the limitations of microscopy and RDTs?*

   A quality assured nucleic acid-based test is the best way to identify all infections in a community.

4. *What is the role of PCR in malaria elimination settings for surveillance and case management, and what are the requirements for quality assurance?*

   For case management purposes, microscopy and RDTs should continue to be used. PCR is likely to be increasingly used for surveillance.

5. *What are the most sensitive and easy to use assays to detect gametocytes and their contribution to transmission, for use in research studies?*

   From a programmatic perspective, there is no need to specifically detect gametocytes. For research purposes, real time quantitative nucleic acid sequence-based amplification (QT-NASBA) or real-time qPCR are the recommended tools.

6. *What is the best screening tool for detection of malaria asymptomatic carriers in airports and at borders?*

   This depends on how screening is conducted and on the local circumstances. If immediate diagnosis is required then an RDT should be used. If the most sensitive tool is required for screening, then a nucleic acid-based test should be used and individuals with a positive test traced subsequently.

7. *Can current serological tests (ELISA) assist in differentiating recent versus old infection?*

   It is not currently possible to differentiate recent from old infections using serological tests, but it is expected that this will be possible in the future.

8. *What are the best diagnostic tools to confirm interruption of transmission, for certification of malaria elimination?*

   More information is needed on how nucleic acid-based test and microscopy results differ in these circumstances. Serology may be useful in areas or populations where no exposure to malaria is expected, and seropositive individuals can then be followed up for further investigation by nucleic acid-based techniques.

9. *What is the role of current serology techniques for malaria diagnosis?*

   None for *P. falciparum*, but serological techniques may be of benefit in identifying individuals with *P. vivax* exposure who could be treated to clear hypnozoites. However, this strategy requires well-characterised cohort studies to demonstrate its impact.

10. *What resources and tools are required to sustain diagnostic capacity in low transmission settings and/or in areas at risk of re-introduction of malaria?*

    Microscopy capacity (quality assured and competency assessed) should be maintained, but preparations should be made for an increasing role for nucleic acid-based methods. The country context will determine whether microscopy capacity should be maintained at large scale in health facilities, or at central referral laboratories.

Annex 2: Suggested modifications to text from selected WHO reports

1. Malaria Elimination: A field manual for low and moderate endemic countries

First programme reorientation: the pre-elimination programme (page 19-20)

From the start of the pre-elimination programme onwards, 100% diagnosis by Giemsa-stained microscopy or a test of equivalent or higher sensitivity and specificity ~~(as opposed to RDT)~~ needs to be phased in, because:

- ~~•~~ it is increasingly important to accurately determine parasite species. ~~and densities detect gametocytes;~~

Programme reorientation has been achieved when cases are limited to clearly defined foci only, and the following changes have been completed:

- all malaria cases are microscopically confirmed and treated according to national policy, including cases diagnosed and treated in the private sector;
- microscopy and nucleic acid-based test quality-assurance systems are fully operational

Interruption of transmission (page 45-46)

In order to be confident that interruption of transmission has been achieved, a number of preconditions must be met. These include:

- high-quality laboratory services to diagnose malaria, based on microscopy or methods of equivalent or higher sensitivity or specificity.

~~The value of sero-epidemiological surveys is limited by the sensitivity of the test methods available. It is also not 100% certain when malaria antibodies are no longer detectable among populations who have previously been exposed to local transmission. All people with positive test results during sero-epidemiological surveys should be fully assessed, including diagnosis by microscopy, treated for current infection, and a full case investigation carried out.~~
"Sero-epidemiological surveys are an important method to confirm the absence of malaria exposure. All people who by virtue of age or location are assume not to have been exposed to malaria but have positive test results during sero-epidemiological surveys should be fully assessed by microscopy or other test of equivalent or higher sensitivity or specificity."

Direct measurements of potential local transmission (page 48)

Direct measurements of potential local transmission include entomological monitoring activities, such as the abundance of vector species, proportion of nulliparous mosquitoes (or other measures indicating physiological age), mapping of risk areas and monitoring resistance of vectors to insecticides. Other direct measurements are specific parasitological surveys with blood slides or ~~rapid diagnostic tests~~nucleic acid-based tests of equivalent of higher sensitivity and specificity~~;~~. ~~s~~Seroepidemiological surveys can be used to evaluate the size of the risk for importation, to identify high-risk immigrants and to evaluate former foci of

transmission.~~; and g~~Genetic characterization may be used to distinguish single-source local infection from imported sources as well as searching for the origin(s) of parasites.

2. Universal access to malaria diagnostic testing: operational manual

Types of tests appropriate for parasitological diagnosis of malaria (page 4-5)

Routine parasitological confirmation of malaria is based on either identification of parasites in blood films examined by light microscopy or detection of parasite antigens with RDTs. Other diagnostic tests, such as nucleic acid-based tests of equal or higher sensitivity and specificity than microscopy~~as polymerase chain reaction (PCR)~~, may be used in certain situations,
such as for

- Distinguishing morphologically similar species (*Plasmodium malariae* and *P. knowlesi*),
- for efficacy testing to distinguish new infections from relapses and recrudescences
- specific elimination and containment ~~projects~~settings

These tests are presently not indicated for the case management of fever.
Serological tests for malaria have no place in the management of febrile patients.

Advanced malaria control and pre-elimination (page 96)

Programmes that have achieved an advanced stage of malaria control and are progressing towards pre-elimination should maximize efforts to ensure universal access to malaria diagnostic testing. Further, the distribution of malaria transmission is often highly heterogeneous within a country, so that a good malaria surveillance system is necessary to guide subnational malaria control strategies.

When the outpatient malaria positivity rate in health facilities is below a certain threshold (e.g. 1%), malaria testing should be restricted to those patients with a high probability of having malaria. This restriction should be extended to all levels of the health system, including communities using simple criteria (for example, 'fever and no pneumonia and no diarrhoea'). In such settings, the strategy of active case detection is used to find and treat all positive febrile cases among people living in the area in which an index case (detected at the health facility) lives. This strategy is easier to implement if there are community health workers who are fully integrated into the health system. It requires the use of microscopy , RDTs or other molecular tests of equal or higher sensitivity and specificity. ~~sensitive diagnostic tools (RDTs with a high panel detection score or quality assured expert microscopy).~~ RDTs have the advantage of permitting immediate treatment on site.

(Note – a future policy statement is recommended on potential treatment of all P. vivax *seropositives for clearance of hypnozoites)*

In focused screening and treatment (screening all people living in a defined geographical area) or mass screening and treatment (screening of an entire community in a broad geographical area) programmes, the people to be detected, and treated if positive, are asymptomatic and generally have a low malaria parasite density. The sensitivity of ~~the malaria tests presently available~~microscopy and RDT are insufficient to detect these subjects, and alternative assays of high sensitivity based on nucleic acid techniques which allow speciation should be used. ~~are needed that are reasonably specific and minimally invasive.~~

3. *Disease surveillance for malaria elimination: operational manual (2012)*

Factors influencing the extent of active case detection undertaken as part of a field investigation (page 12)

While ~~PCR~~ nucleic acid-based tests can be used to detect asymptomatic infections in some settings, the potential programme value of detecting low-density infections that are microscopy-negative but PCR-positive is unclear.

Case definition (page 7)

Case of malaria (as defined in elimination programmes): a case in which, regardless of the presence or absence of clinical symptoms, malaria parasites have been confirmed by quality controlled laboratory diagnosis.

Even when rapid diagnostic tests are used for initial patient management, clinics should make a microscopy slide or collect adequate sample for nucleic acid-based testing of equivalent or higher sensitivity and specificity, at the same time for subsequent confirmation of the diagnosis at a nearby reference laboratory. Cases with a positive rapid diagnostic test but no slide taken at the time of initial contact should be investigated in the same way as cases confirmed by microscopy.

4. *Community-based reduction of malaria transmission (2010).*

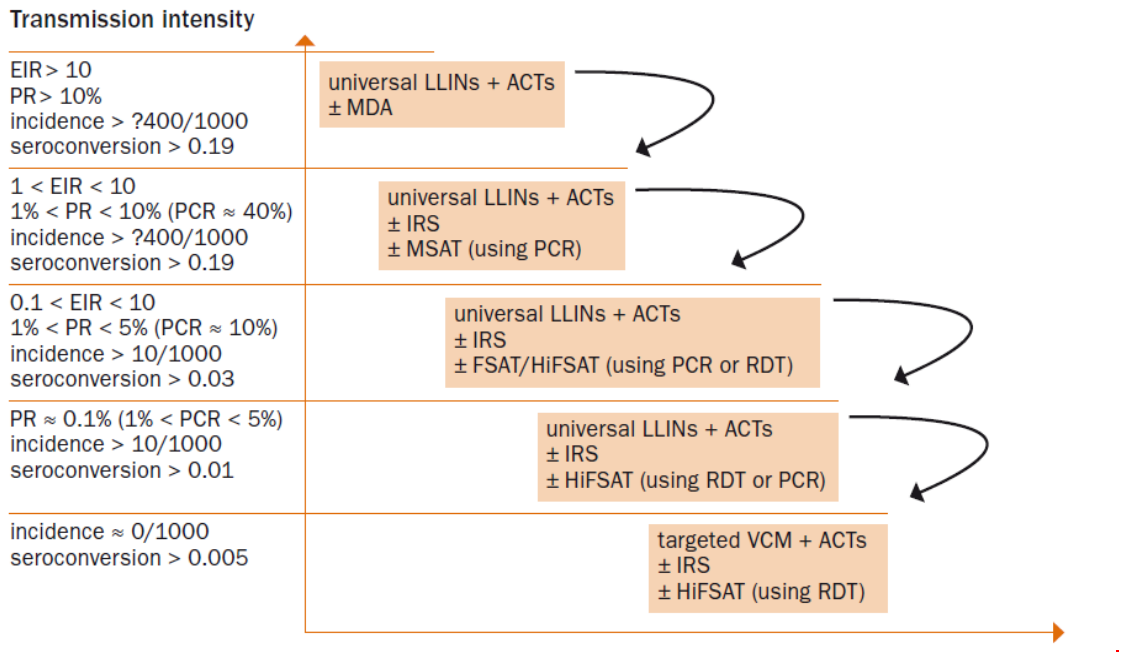
Intervention packages for community-based reduction of malaria transmission (page 15)

For the management of symptomatic cases, RDT and microscopy are sensitive enough at any level of malaria transmission~~, and PCR is not necessary~~. The most sensitive test for screening an asymptomatic population is a nucleic acid-based test~~presently PCR~~.

PCR has several logistic limitations, as mentioned above, and the experience in Pailin (2008-2009) concluded that MSAT with PCR should be replaced by FSAT. ~~In areas where transmission has been low for a long time and the reservoir of asymptomatic carriers is almost non existent, microscopy and RDT might be sufficient.~~ Even in areas of very low levels of tranmission there may be a reservoir of asymptomatic carriers and the most sensitive tools should be deployed to identify them.

Designing the intervention (page 19)

Diagram is based on many assumptions and requires review of the current evidence



Throughout the diagram, replace PCR with 'nucleic-acid based test'. RDT should be removed throughout the diagram.

Community screening and case management (page 27)

• If MSAT or FSAT are considered to be the appropriate initial transmission reduction strategies ~~on the basis of local transmission intensity~~, screen each member of the community with a RDT, regardless of age, gender or the presence of malaria symptoms. Consult the local health facility to determine the respective roles of facility personnel and community health workers in these activities. Treat anyone with a positive malaria RDT (regardless of symptoms) with the first-line antimalarial medicines provided, in line with the national treatment policy (taking the necessary precautions for children weighing < 5 kg, pregnant women in their first trimester and people with a known allergy to antimalarial medicines).

Case management, active case detection and highly focused screening and treatment

• Perform an RDT on all febrile patients (suspected malaria) and perform a more sensitive nucleic acid-based test on all household members of patients with a positive RDT (household active case detection), providing treatment on the basis of that nucleic acid-based test.
• Treat all persons with a positive RDT in line with national policy.
• Undertake active household case detection for malaria patients identified at the local health facility and drug shops.

Evaluating community-based reduction of malaria transmission (page 35)

Although none of the component interventions of community-based reduction of malaria transmission is new, the package has not been widely tested or implemented. Therefore, a basic evaluation framework is recommended. The main effect measure that is proposed is the difference in parasite prevalence 12 months after the start of the programme when compared with the baseline. This measure would be obtained with RDTs, blood films or ~~PCR~~ nucleic acid-based test on a simple random sample of people in the community, who would also respond to a household survey on LLIN ownership and use and risk factors for malaria. Extended follow-ups with the same technique would be done at 18 and 24 months.

Annex 4: Key references providing evidence to support new recommendations

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10. Bousema T, Drakeley C: **Epidemiology and infectivity of Plasmodium falciparum and Plasmodium vivax gametocytes in relation to malaria control and elimination.** *Clinical Microbiology Reviews* 2011, **24:**377-410.

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GLOBAL MALARIA PROGRAMME

WHO policy recommendation on malaria diagnostics in low transmission settings

March 2014

In recent years, the application of nucleic acid amplification (NAA)-based diagnostic tools to detect malaria in the context of epidemiological surveys and in research endeavours has increased significantly. Many different assays are available with a superior diagnostic performance to microscopy and rapid diagnostic tests. Based on a recent evidence review,[1] the following are recommendations on the role of molecular diagnostic tests for malaria in low transmission areas.[2]

Recommendation 1

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

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

Recommendation 3

Submicroscopic *Plasmodium falciparum* and *P. vivax* infections are common in low as well as 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.

Continued

1 Report available on the WHO-GMP website at the following URL: www.who.int/malaria/mpac/mpac_mar2014_diagnosis_low_transmission_settings_report.pdf

2 Defined according to WHO (2012) *Disease surveillance for malaria elimination: an operational manual* www.who.int/malaria/publications/atoz/9789241503334/en/

Recommendation 4

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

Recommendation 5

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

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

Recommendation 6

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