

**WHO Evidence Review Group on  
Malaria Diagnosis in Low Transmission Settings**

WHO Headquarters, Geneva, 16-18 December 2013

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**Meeting Report**

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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.

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**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/ $\mu$ l). 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 parasites and 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  Limited temporal variation  Limited geographical variation	Cases and deaths less common distributed according to mosquito bite exposure  Variable within and between years Risk of epidemics  Geographical heterogeneity Concentrated in marginal populations	Cases sporadic  Imported cases may be high proportion of total  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/ $\mu$ l) 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  $\mu\text{l}$  blood ( $\text{p}/\mu\text{l}$ ) while the expert microscopist will struggle to detect regularly infections  $<20 \text{ p}/\mu\text{l}$  (Chiodini, unpublished). The limit of detection for RDTs is generally in the order of  $100 \text{ p}/\mu\text{l}$ , while lab-based PCR methods generally have published limits of detection of  $<5 \text{ p}/\mu\text{l}$  [22, 23].

Diagnostic technique	Operational characteristics	Performance <sup>1</sup>	Cost <sup>2</sup>	References
<b>Nested PCR</b>	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 <i>Plasmodium</i> species. Hands-on time 3 hours to result, total time 10 hours.	\$1.5-4.0 per sample, \$500-5000 for equipment	[24]
<b>Multiplexed PCR</b>	Simultaneous, multiplex PCR to detect the presence of multiple <i>Plasmodium</i> 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]
<b>Quantitative PCR</b>	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 <i>P. falciparum</i> detection. 60 minutes hands-on time to result, total time 2.5 hours.	\$4-5 per sample, >\$20,000 for equipment	[29-32]
<b>LAMP</b>	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, <i>P. falciparum</i> and <i>P. vivax</i> . 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]
<b>QT-NASBA</b>	Assay includes a reverse transcriptase step, less inhibition than PCR. Isothermal method. Can be used for gametocyte quantification. Detects all four <i>Plasmodium</i> 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]

<sup>1</sup> 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.

<sup>2</sup> 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<sup>3</sup> 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.

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<sup>3</sup> 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/ $\mu$ l 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/ $\mu$ l 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 50 $\mu$ l 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  $R_c > 1$  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  $R_c > 1$ .

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  $R_c > 1$ , 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  $R_0 < 1$  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  $\mu\text{l}$ . The assay performed best when using 5  $\mu\text{l}$  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](http://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  $\leq 2$  parasites/ $\mu$ l.
- Need for a sample volume of not more than 50 $\mu$ l 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 ( $R_0$ ,  $R_c$ ), 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. 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~~ 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 transmission 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

### Transmission intensity

EIR > 10  
PR > 10%  
incidence > 7400/1000  
seroconversion > 0.19

universal LLINs + ACTs  
± MDA

1 < EIR < 10  
1% < PR < 10% (PCR ≈ 40%)  
incidence > 7400/1000  
seroconversion > 0.19

universal LLINs + ACTs  
± IRS  
± MSAT (using PCR)

0.1 < EIR < 10  
1% < PR < 5% (PCR ≈ 10%)  
incidence > 10/1000  
seroconversion > 0.03

universal LLINs + ACTs  
± IRS  
± FSAT/HiFSAT (using PCR or RDT)

PR ≈ 0.1% (1% < PCR < 5%)  
incidence > 10/1000  
seroconversion > 0.01

universal LLINs + ACTs  
± IRS  
± HiFSAT (using RDT or PCR)

incidence ≈ 0/1000  
seroconversion > 0.005

targeted VCM + ACTs  
± IRS  
± HiFSAT (using RDT)

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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***Annex 5: List of participants***

Michael AIDOO  
Centers for Disease Control and Prevention, Atlanta  
U.S.A

Ruth ASHTON  
Rapporteur  
London  
UNITED KINGDOM

Philip BEJON  
KEMRI Wellcome Trust Research Programme  
Kenya Medical Research Institute, Kilifi  
KENYA

David BELL  
Independent consultant, Geneva  
SWITZERLAND

Teun BOUSEMA  
Department of Immunology and Infection  
London School of Hygiene & Tropical Medicine, London  
UNITED KINGDOM

Lydie CANIER  
Molecular Epidemiology Unit  
Institute Pasteur of Cambodia, Phnom Penh  
CAMBODIA

Qin CHENG  
Australian Army Malaria Institute, Enoggera  
AUSTRALIA

Peter CHIODINI  
Department of Clinical Parasitology  
Hospital for Tropical Diseases, London  
UNITED KINGDOM

Jackie COOK  
Zanzibar Malaria Research Unit Karolinska Institutet, Zanzibar  
UNITED REPUBLIC OF TANZANIA

Valerie D'ACREMONT  
Swiss Tropical and Public Health Institute, Basel  
SWITZERLAND

Chris DRAKELEY  
Department of Immunology and Infection  
London School of Hygiene & Tropical Medicine, London  
UNITED KINGDOM

Brian GREENWOOD (Co-Chairperson)<sup>4</sup>  
Department of Infectious and Tropical Diseases  
London School of Hygiene and Tropical Medicine, London  
UNITED KINGDOM

Iveth GONZALEZ  
Foundation for Innovative New Diagnostics, Geneva  
SWITZERLAND

Mallika IMWONG  
Faculty of Tropical Medicine, Mahidol University  
Molecular Tropical Medicine and Genetics Department, Bangkok  
THAILAND

Edwin KAMAU  
Malaria Drug Resistance Programme  
US Army Medical Research Unit-Kenya, Nairobi  
KENYA

Paul LABARRE  
PATH  
Director Project DIAMETER, Seattle  
U.S.A

Kevin MARSH (Co-Chairperson)<sup>4</sup>  
KEMRI Wellcome Trust Research Programme, Kilifi  
KENYA

Sylvia MEEK<sup>4</sup>  
Malaria Consortium, London  
UNITED KINGDOM

Ivo MUELLER  
Infection and Immunity Division  
Walter and Eliza Hall Institute of Medical Research, Melbourne  
AUSTRALIA

Nyasatu NTSHALINTSHALI  
Clinton Health Access Initiative  
Southern Africa Malaria Elimination Support Team, Mbabane  
SWAZILAND

Lucy OKELL (via Skype)  
Imperial College London  
Faculty of Medicine, School of Public Health, London  
UNITED KINGDOM

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<sup>4</sup> Also member of the Malaria Policy Advisory Committee

Christian F. OCKENHOUSE  
PATH  
Malaria Vaccine Initiative, Washington  
U.S.A

André Lin OUEDRAOGO  
Centre National de Recherche et de Formation sur le Paludisme  
Department of Biomedical Sciences, Ouagadougou  
BURKINA FASO

Mark PERKINS  
Foundation for Innovative New Diagnostics, Geneva  
SWITZERLAND

Georges SNOUNOU  
INSERM UMR-S 945, Paris  
FRANCE

Venkatachalam UDHAYAKUMAR  
Malaria Branch, Division of Parasitic Diseases  
Centers for Disease Control and Prevention, Atlanta  
U.S.A

#### **OBSERVERS**

Melto J. ELIADES  
MalariaCare/PATH, Washington  
U.S.A

Heidi HOPKINS  
Foundation for Innovative New Diagnostics, Geneva  
SWITZERLAND

Kathy TIETJE  
PATH, Seattle  
U.S.A



**WHO SECRETARIAT**

Hoda ATTA, WHO Regional Office for EMRO

Andrea BOSMAN, Global Malaria Programme

Eva CHRISTOPHEL, WHO Regional Office for WPRO

Jane CUNNINGHAM, Global Malaria Programme

Josephine NAMBOZE, WHO Regional Office for Africa

Robert NEWMAN, Director, Global Malaria Programme

Piero Luigi OLLIARO, Special Programme for Research and Training in Tropical Diseases

John REEDER, Director, Special Programme for Research and Training in Tropical Diseases

Pascal RINGWALD, Global Malaria Programme

Vaseeharan SATHIYAMOORTHY, Initiative for Vaccine Research