Title: MALVAC 2010: Measures of Efficacy of Anti-malarial Interventions against Malaria Transmission, 15-16 November 2010 in Geneva, Switzerland

Authors: M. Pinder\textsuperscript{1}, V. S. Moorthy\textsuperscript{2,3}, K. Mendis\textsuperscript{4}, G.V. Brown\textsuperscript{5} on behalf of the WHO MALVAC committee

Affiliations:
1 MRC Laboratories, PO Box 273, Fajara, The Gambia
2 Initiative for Vaccine Research, World Health Organization, 1211 Geneva 27, Switzerland
3 Nuffield Department of Clinical Medicine, University of Oxford, John Radcliffe Hospital, Oxford, OX3 9DU, UK
4 Independent Consultant, Colombo, Sri Lanka
5 Nossal Institute for Global Health, Centre for Clinical Research Excellence in Infectious Diseases, University of Melbourne, Carlton, Victoria 3010, Australia
Abstract

WHO Initiative for Vaccine Research convened a meeting of experts to evaluate the potential assays and trial designs that should be applied to measurement of reduction of transmission of *P. falciparum* malaria. Though the meeting focussed particularly on the question of evaluation of malaria transmission-blocking vaccines (i.e. those vaccines whose biological target acts to prevent transmission from humans to mosquitoes), the discussions encompassed many wider issues of relevance to evaluation of all anti-malarial interventions from the perspective of transmission reduction.

Standardized measures of malaria transmission have not yet been agreed and appropriate measures may differ between low and high transmission areas. Measures of malaria transmission can be divided into four broad classes: epidemiological (incidence of new human infections), entomological (estimating new human infections by mosquito measures), assays of transmission from humans to mosquitoes (such as membrane feeding assays which estimate how many mosquitoes become infected from human blood) and surrogate serological and molecular measures such as ELISA and PCR. Greater standardization is required and the relative applications and pros and cons of the different measures are discussed in detail in this report. New, sensitive assays need to be made high-throughput, low-cost and useable in the field if they are to play a role in malaria surveillance in future.

Clinical and regulatory pathways for pre-clinical and clinical development, licensure and access for transmission-reducing malaria vaccines require establishment over coming years. At this meeting potential accelerating factors were discussed. Design and conduct of a cluster randomized trial would be a major, complex undertaking and so every avenue should be explored to validate surrogate assays of transmission reduction in discussion with regulatory authorities. In addition, a discussion of the roles for modelling of impact of anti-malarial interventions are outlined in this report.

The success of malaria control as transmission drops will depend to a large extent on identifying and effectively targeting remaining hotspots of malaria transmission, at village and district levels. Remarkably little recent data is available on transmission-related epidemiology in different settings that characterises human infectious reservoirs, tracks malaria parasite infection and exposure spatiotemporally and quantifies vectorial capacity. These issues represent a priority R&D agenda for malaria control and evaluation of transmission reduction.
**Introduction**

The World Malaria Report 2010 documented the major progress being made with malaria control in several countries including some in Africa. The total number of malaria cases globally has been estimated at 225 million for the year 2009 and the estimated annual malaria-related deaths remains above 780,000. These estimates allow for the paucity of data for many African malaria-endemic countries.

Current routine surveillance measures for malaria focus on clinical cases of malaria and malaria-related mortality reported to the WHO by member states. In recent clinical trials, measures of efficacy of preventive malaria interventions, such as vaccines and intermittent preventive therapy, have focused on clinical malaria. Nevertheless reduction in transmission remains the fundamental basis of malaria control. Indeed, where transmission intensities are low to moderate, efforts have been primarily directed towards lowering transmission, and these have led to a reduction in morbidity and mortality. In areas of intense transmission, however, efforts have been largely focussed on reducing morbidity and mortality rather than lowering transmission. This is because the feasibility of reducing transmission at such high intensities was questioned, and its potential impact unclear. Yet, it is increasingly apparent that some currently available anti-malarial interventions lead to a reduction in transmission even in these highly malarious areas.

As new tools for malaria control are developed, it will be desirable to quantify their impact on malaria parasite transmission. In moderate to high transmission settings, such measurements will provide valuable additional information on the potential utility of new malaria interventions. In low transmission settings, the feasibility of elimination may become a desired assessment. Many in the global malaria R&D community now aspire to develop new and improved tools to make planning for global eradication a possibility. In this framework, transmission reduction becomes a key metric in the measurement of impact of new tools and combinations of intervention methods.

Malaria vaccines, in addition to reducing morbidity and mortality, will also impact parasite transmission; transmission blocking and pre-erythrocyte vaccines directly, and possibly asexual blood stage vaccines more indirectly. Measuring changes in transmission, therefore, should be seen as a highly desirable goal in the context of all antimalarial interventions, including malaria vaccines, as it allows a better understanding of the interaction between the different types of malaria interventions (mosquito control, treatment, vaccination) and their combined impact, and may facilitate better evidence-based decision-making for optimal integration of malaria control interventions and strategies for a given setting.

The evaluation of malaria vaccines from the perspective of reduction in transmission was the subject a WHO Initiative for Vaccine Research Scientific Forum Meeting held in Geneva in November 2010, bringing together 70 vaccine researchers, clinical epidemiologists and field trialists with funders and representatives from regulatory agencies. The objectives of the meeting were to evaluate the current methods for measurement of malaria parasite transmission and consider approaches to measure reduction in transmission primarily through clinical trials. A session on mathematical
modeling was included. See Box 1 for an outline of the meaning of acronyms related to malaria transmission-blocking vaccines (TBV). The current meeting related mostly to development of TBV, though aspects of this meeting report are also highly applicable to evaluation of other vaccine and non-vaccine interventions from the perspective of transmission reduction.

This technical meeting took into account the contribution of the extensive malaria parasite eradication R&D agenda-setting process and a previous gathering hosted by PATH Malaria Vaccine Initiative on Transmission-Blocking Vaccines in Washington DC in June 2010.

Projections of the expected impact of interventions in the framework of transmission

The elements of R0 (see Box 2) that can be manipulated by intervention are the density of mosquitoes, the proportion of infected mosquitoes, their human biting rate, the daily survival rate of the mosquito and the magnitude of the parasite pool in humans. Figure 3 outlines how different intervention modalities affect these different factors. A vaccine that impacts transmission through an effect on any life-cycle stage will primarily act to reduce the proportion of infected mosquitoes. If the vaccine were to reduce the number of parasites within mosquitoes (e.g., by reducing oocyst density) without reducing the proportion of infected mosquitoes it is hypothesized that this may also reduce transmission though there is little data to support this. Reductions in oocyst density would almost certainly reduce the number of parasite strains circulating, though again there is no field data to support this.

Assays for evaluation of reduction in transmission from human to mosquito

The availability of assays that are predictive of infectivity of humans for mosquitoes is an important asset in TBV development. Commonly used assays for this purpose rely on feeding of susceptible mosquitoes on Plasmodium-infected blood and measuring both the proportion of infected mosquitoes and the number of oocysts per mosquito. Robert Sauerwein of Radboud University of Nijmegen Medical Centre described the Standard Membrane Feeding Assay (SMFA) which uses sera or purified immunoglobulins (IgGs), in vitro cultured gametocytes and well-established laboratory strains of Anopheline mosquitoes (Vander Kolk, M 2006 Parasitology) to assess the effect of immune responses on malaria parasite transmission. Antibody concentrations to Pfs48/45, a gametocyte antigen, determined by ELISA correlate reasonably well with the SMFA(1). Challenges of the assay, however, include its limited sensitivity since it requires dilution of the serum, the considerable variation in oocyst number between experiments (see Carole Long's presentation) and the limited number of cultured parasite strains which produce infectious gametocytes in vitro. Despite these shortcomings, the SMFA has proven to be a useful tool in preclinical and clinical studies and the Sauerwein laboratory has set, as a Go/No-Go criteria, >75% oocyst reduction in >70% of immunized animals to advance its Pfs48/45 pre-clinical vaccine candidate to human clinical trials. Selection of this threshold is an important issue for the TBV field, because the effect of an
individual result in the SMFA has yet to be correlated with impact on transmission in the field.

An alternative assay is a direct membrane feeding assay (DMFA) which uses blood samples drawn from humans naturally infected with malaria parasites as a source of gametocytes. This bioassay is designed to measure infectiousness of gametocyte carriers. Replacement of autologous plasma with sera from non-immune human subjects serves as a control for determining the inherent infectivity of the gametocytes in the blood sample. One of the major advantages of the DMFA is it allows an assessment of field-derived parasite isolates for infectivity.

A third assay is the direct feeding assay (DFA) in which laboratory-reared mosquitoes are fed directly on naturally exposed individuals. Historically this assay led to the early descriptions of transmission blocking activity in some naturally exposed individuals. Because wild-caught mosquitoes may transmit adventitious agents, DFA with laboratory-reared mosquitoes is the only assay involving direct feeding of mosquitoes on humans. During the meeting some participants from endemic countries raised concerns about use of DFA in two circumstances. Firstly DFA with wild-caught mosquitoes is now considered ethically unacceptable by several ethical committees, due to the risk of adventitious agents. Secondly concerns were raised about performance of DFA with paediatric trial subjects even with laboratory-reared mosquitoes, with many feeling this is not justifiable. Should studies involving DFA with laboratory-reared mosquitoes be proposed in adults, emphasis should be placed on detailed informed consenting.

Other non-clinical assays include rodent model systems using transgenic parasites such as \textit{P. berghei} parasites that express a \textit{P. falciparum} sexual stage gene.

Sauerwein also briefly described transmission reducing antibodies (TRA - see figure 4) to these antigens in those living in malaria endemic areas ie naturally acquired functionally active transmission-blocking antibodies (see Dr Ouedraogo's presentation). The range of activity found is highly disparate, a few have strong natural TRA, while some show no blocking effect and others have moderate levels. In low level transmission areas, TRA are highest at the end of the transmission season, suggesting that naturally induced TRA are rapidly induced and relatively short-lived. It has also been reported that these TRA decrease with age, possibly due to the shorter duration of asexual stage infections in older children and adults. Limited research has been conducted into this area, as such, what effect TRA may have and how predictive these assays may be on malaria parasite transmission are presently unknown.

**Status of Nijmegen Pfs48/45 vaccine project, The Netherlands**

Sauerwein continued with an update on the target molecule Pfs 48/45, a member of the 6-cys family which plays a key role in the fertility of male gametocytes. Monoclonal antibodies to Pfs48/45 have transmission-blocking activity. The construct Pf10C-MBP, which represents a portion of Pfs48/45 coupled to maltose binding protein and co-expressed with chaperones in \textit{E coli}, induces transmission-blocking activity in rodents. Genetic polymorphism occurs for this candidate but is less extensive than for most blood-
stage candidate antigens. Pf10C-MBP is currently undergoing cGMP development and adjuvant selection on a path towards a Phase 1a trial in the Netherlands. In addition, Sauerwein and other colleagues are selecting TBV-testing sites with annual EIRs in the range of 1-20 where gametocyte carriage of the populations and naturally acquired antibody are being determined.

The discussion after this presentation raised points on the need to trial TBV in areas with appropriate levels of transmission—if the transmission rates are too low, the size of the trials may become too large to be feasible, whereas if the transmission intensity is too high, a biologically meaningful effect under conditions of lower transmission intensity may go undetected. Other discussion points included consideration of the infectious reservoir for such trials and tools predictive of infectivity of humans to mosquitoes (e.g., neither asexual nor sexual stage parasitaemia by microscopy appear to be sufficient to adequately predict infectivity). There was also discussion as to whether the Go/No-Go criteria for advancing the development of a vaccine candidate based on reduction of oocyst density (as opposed to complete inhibition of oocyst development) was sufficiently stringent; some raised the possibility that the specified levels of oocyst reduction may have clinically significant effects depending on the transmission setting of interest. Robert Sinden of Imperial College commented on modeling by his group of TBV impact at different EIR levels: at 10-20 EIR, a TBV able to give a 75% reduction of oocyst density would have an effect on transmission in his modeling work. In the end, no consensus was reached on the relationship between reduction in oocyst density and effect on EIR.

In response to a question about how dependent the assay is on the mosquito vector used, Sauerwein noted that two strains of *Anopheles gambiae* have been used in DMFA at Nijmegen and the results are highly comparable; that said, Sauerwein pointed out that this comparability in a laboratory setting may not reflect the mosquito vector differences found in the field.

**Status of TBV vaccine projects at the Laboratory of Malaria Immunology and Vaccinology (LMIV), NIH, USA**

Patrick Duffy presented on the clinical development of Pfs 25 vaccines. He reviewed the history of malaria transmission blocking vaccines pursued at the U.S. National Institutes of Health and outlined the future directions for the group, now renamed the Laboratory of Malaria Immunology and Vaccinology (LMIV). Their key TBV strategies centre on the sexual/mosquito stage antigen Pfs 25 and they envision central roles for combination vaccines including CSP (circumsporozoite protein) and Pfs25, with other antigens such as Pfs230, Pfs28, and Pfs48 in the pipeline. The LMIV also plan to work on assay standardization and qualification as well as the important issue of formulation, either with new adjuvants or via conjugation to other carrier proteins to enhance immunogenicity. He reminded the group that conjugation of Pfs25 to the outer membrane protein complex (OPMC) of *Neisseria meningitidis* had previously elicited high levels of antibody lasting approximately 2 years in rhesus monkeys(2). Current conjugates with Pfs25 are focusing on recombinant exoprotein A from *Pseudomonas aeruginosa* (EPA), which has been used extensively in children and has a US govt. patent. A Phase 1 trial of Pfs25 is
anticipated in 2011. His group is proposing that progression from Phase 1a to Phase 1b will require transmission blocking activity of >50% reduction in oocyst count in >50% of vaccinees. If these conditions are met, a Phase 1b trial will be conducted in Mali and this must achieve >80% reduction in oocyst count in >80% of volunteers with antibody sustained for 9 months for this formulation to progress. Discussion included the approach to the combination of antigens, and problem of defining Go/No-go criteria because of unknown relationships between partial efficacy in a heterogeneous group of people and anticipated effect on transmission.

**Trial design to capture indirect effects in malaria**

Paul Milligan presented the range of different study designs and endpoints that could be used for testing VIMT (not only TBV) in the field, using cluster randomised trials. Important features of these study designs are to measure (1) the direct effect of protection on vaccinated subjects (for those vaccines that contain components targeting the pre-erythrocytic and/or erythrocytic stages), (2) the indirect effects of vaccine coverage on protecting vaccinated and unvaccinated subjects (i.e. a reduction in force of infection at the community level) and (3) the increasing age at time of new infection in study subjects. He noted that the indirect effects would vary by population and transmission setting.

He proposed that the preferred design should be to stratify clusters by 1 or 2 strata (e.g. geographical position or urban/rural), rather than matching control and intervention clusters. One alternative approach would be to use a wedge-shaped design that may be operationally more practical. A major concern of these studies is to reduce contamination of clusters by movement of infective mosquitoes and/or people from outside the study area. Contamination by infective mosquitoes could be reduced by having a buffer area around a central core where study subjects are sampled; he suggested a buffer zone of 2-4 km.

Comprehensive baseline information in all vaccine testing sites is required, describing the local ecology of transmission, including population movement and heterogeneity of transmission. It was suggested that several clinical endpoints need to be measured related to clinical incidence of disease and parasite infection. It will be particularly important to identify malaria transmission ‘hot spots’ in the study areas. This could be done using a number of different approaches including (1) incidence of infection or clinical malaria in infants, (2) mosquito exposure, (3) parasite prevalence, (4) serology and (5) remote sensing.

There was discussion about the approach to Long-Lasting Insecticidal Nets (LLIN) coverage in such trials with some feeling that LLIN coverage should be facilitated. It was pointed out that the previous bednet trials of the 1990s represent the best precedent for design of future trials to evaluate indirect effects. However, the limited size of cluster units and the absence of buffer zones in randomized control trials may have underestimated the efficacy of insecticide impregnated bed nets and improved trial designs are now possible going forwards.
Teun Bousema presented his highly pertinent data from Mali and Tanzania on heterogeneity in malaria transmission(3). It is well known that malaria transmission is highly variable by place and time at low levels of transmission. His data, however, illustrates that transmission also varies in high transmission endemic areas: over 75% of mosquitoes are caught in the same 40% of households, be it the dry or the wet season. This heterogeneity is also observed for other malaria parasite parameters, including incidence of infection in infants and parasite seropositivity rates.

There was an inconclusive discussion about targeting hotspots for malaria control; possible geographical shifts in hotspots over time was raised as a major concern.

Endpoints in individually randomized trials to measure efficacy of interventions on transmission

David Kaslow presented the case for considering alternative methods of assessing vaccine efficacy that do not rely on cluster randomized trials but rather take an "accelerated approval" approach to assessing the biological activity of a transmission blocking vaccine in humans. His proposal is based on the strategy of establishing whether a vaccine is reasonably likely to have a protective effect on transmission by showing that a TBV has an effect on a surrogate endpoint, that is likely to predict clinical efficacy, in two or more, independent, multi-center, double-blinded, randomized, controlled Phase 2b studies powered to demonstrate robust superiority. He emphasized that this surrogate endpoint (e.g. oocyst prevalence following membrane feeding) must be shown to correlate with a desired clinical endpoint and must capture the net effect of the intervention on the clinical efficacy endpoint. Criteria for acceptability of the surrogate endpoint include that it measures (1) the principal mechanisms which determine clinical efficacy, (2) the positive and any negative effects of the vaccine, (3) the overall level of protection in a community and (4) strength and durability of the effect.

Dr Kaslow’s strategy makes several explicitly stated assumptions including that antibodies are the main mediators of efficacy, that direct feeds of mosquitoes on humans are acceptable in a given setting and that the Phase 1 and 2a trials show no safety concerns. Initial screening would take place using the SMFA to determine whether antibodies are effective at preventing oocyst development and the prevalence of oocysts in mosquitoes. This would be followed by direct feeding experiments of mosquitoes on vaccinees to provide indirect evidence that vaccination will reduce malaria parasite transmission in the field.

The audience was asked to consider whether a surrogate marker of clinical protection, such as the SMFA, would suffice to demonstrate clinical protection rather than continue with measuring protective efficacy based on demonstrating a reduction in new infections or morbidity. Provisional approval or a decision to include a TBV in a multi-component vaccine based on Phase 2b studies of the TBV alone to show robust superiority of the candidate over the unvaccinated state would be followed, in a timely manner, by one or more clinical trials that would validate whether the TBV truly provides meaningful benefit on tangible measures of clinical benefit, most likely by Phase 4 effectiveness (as opposed to efficacy) trials. The discussion that followed included a focus on the need to
demonstrate the link between any proposed analytically (but not clinically) validated surrogate markers and the clinical effects of the vaccine. This was also expressed by Ralph Leblanc of US FDA from a regulatory perspective. The consensus was that a formal evaluation of effect of vaccine on transmission was required. Selection of sites where effectiveness could be demonstrated was critical: if studied in a setting where transmission is too high, effectiveness in a lower transmission setting may not be detected, if studied in a setting where transmission is too low, effectiveness may not be measurable.

**Entomological measures of transmission**

Marc Coosemans of the Institute of Tropical Medicine of Antwerp (Belgium) discussed entomological measures for evaluating the impact of interventions on transmission. Personal protection is achieved by individual use of ITN, but community protection can occur when there is a decrease of transmission by a mass killing effect on the vector population which can only occur with a coverage of ITN or IRS above a certain threshold level. This is important to consider in the design of cluster randomized trials as contamination from intervention areas into control ones can result in under-estimation of efficacy of the vector control tools under examination.

Two key measures of transmission can be applied to evaluate the impact of interventions: namely, the entomological inoculation rate (EIR) by unit of time and the vectorial capacity (C), which estimates the daily rate of potential transmission. These measures can be defined mathematically:

\[
C = m a^2 p^n / \log_e p \\
EIR = ma \cdot s
\]

Where, \( ma \) is the human biting rate and \( s \) the sporozoite rate, i.e. the infection rate in mosquitoes, both of these can be estimated by ELISA or dissection of salivary glands. Vectorial capacity also requires estimation of the daily survival of the vectors (\( p \)) and the number of days between the infective blood meal and the presence sporozoites in the salivary glands of the vector (extrinsic incubation period, \( n \)).

Human landing collections (HLC) are the gold standard for estimating human biting rate and they also allow for the simultaneous capture of mosquitoes. The technique demands close supervision of very intensive, skilled work, requiring at least two workers to cover a night shift. This technique is mostly of benefit where transmission is high. Limitations of this method include the observation that subjects vary in attractiveness to mosquitoes, and recent ethical concerns about exposure to known and unknown adventitious agents transmitted by feral mosquitoes. The latter has led to cessation of HLC in some settings, with some groups shifting to use of light traps. These estimations of human biting rates require in-depth knowledge of the local vectors to adapt the collecting methods for the different biting behavior of vector species, for example indoor versus outdoor biting behaviors, time of biting, and interrupted feeding times. As result of wide scale use of
LLIN the proportion of transmission occurring outdoors and before sleeping time has increased in many settings (4, 5) and is often not well documented.

Light traps (LT) can also be used for monitoring human biting rates. LTs may provide a representative sample of the mosquito populations, as long as the host close to the LT is fully protected by a bed net. LT are also less labour intensive than HLC and can provide many data points. Limitations of LTs include lack of reliability for assessing outdoor biting behavior and absence of data on biting times. Moreover trap efficiency is not always density independent and mosquito composition (species, sporozoite index, etc) may be different from HLC, showing that validation (as compared to HLC) of the trapping method should been done in each setting(6).

Infection rates in mosquitoes are commonly measured using an ELISA specific for sporozoites. Coosemans presented results from 6 published studies and new datasets in which sporozoites detected by ELISA were not confirmed by a Plasmodium specific PCR indicating a high level of false positivity particularly in zoophilic mosquitoes. This issue needs further examination. Collection source is critical depending on whether transmission occurs inside (endophilic) or outside (exophilic) dwelling houses (e.g. (7)). Pyrethrum spray catches of indoor resting vectors were raised as a means of estimating vector infection rates for highly endophilic species in specific settings, but it was also pointed out that the available methods can not fully account for non-random distribution of vectors within a village. The average flight distance of the common vectors is about two kilometres.

The minimum EIR thought to be measurable with reliability is about 5-10. If EIR are to be compared in intervention studies then measurements must be taken over a long period as there can be high variability between years.

**Estimating infectiousness from infection rate in wild caught mosquitoes of different ages**

As discussed throughout the meeting, measuring individual infectiousness in humans is very imprecise as 1) gametocyte density correlates poorly with infectivity 2) the direct and membrane feeding assays are not well standardized. An alternative is to measure infection rates in the wild caught vectors in natural transmission situations. Steve Lindsay of LSHTM then gave a short presentation on behalf of Joe Lines on measuring infectiousness (and potentially impact on this) using infections rates and age grading in wild caught mosquitoes. Sporozoite rates, and the potential for transmission, are thus dependent on the initial infectiousness and average age/survival times within a given mosquito population. Measuring mosquito age, however, is not simple. The accepted methods rely on micro-dissection. The Detinova method determines parity by the structure of ovaries. In the Polovodova method a segment of the ovary is dissected and the number of “beads” corresponds to the number of blood meals taken and hence age. Both methods are labour intensive and require large numbers of mosquitoes carefully transported from field to laboratory. Newer methods use gas chromatography of cuticular hydrocarbons to age mosquitoes but these are not yet reliable. A method based on similar principles of comparing infection rates in mosquitoes of different ages uses delayed
oocyst rates in mosquitoes caught by different methods i.e. that have survived through differential numbers of feeding cycles.

In addition to age and lifespan, there is a need to ensure that the vector population sampled is relevant to transmission. Reliance on indoor captures as the sole method for assessing entomological measures of transmission may be misleading; that said, there is as of yet no reliable outdoor trapping method.

In the discussion that followed, there was a debate about the relevance of mosquito survival (a component of vectorial capacity) to vaccine interventions. Many felt the primary focus for clinical trials of vaccines should be on clinical endpoints that measure incidence of human infection with entomological endpoints falling into the category of secondary or exploratory endpoints.

**Overview of assays for measurement of infection or parasite exposure in humans**

Chris Drakeley then gave a summary of other assays with applications for measuring prevalent infection, gametocytaemia and parasite exposure. These include PCR and RT-PCR, QTNASBA and LAMP (Loop-attenuated iso-thermal amplification); these techniques need to be compared and standardized. Very low levels of transmission can be assessed by population level serology, whether malaria is epidemic or at pre-elimination levels. Again there is a range of techniques including ELISA and Luminex. These techniques can monitor changes in malaria parasite transmission over time, though the data on assay results in relation to long-term changes in exposure is limited for some assays. Techniques to measure exposure of humans to *An. gambiae* saliva peptides have been applied to measure the impact of ITN. Entomological measures such as EIR have most utility at moderate to high transmission (EIR>10) and serological measures are of most utility at low transmission. Measures of asexual infection are responsive to intervention in the mid range of transmission as they become saturated at high transmission and are non-discriminatory at low transmission.

The discussion included a question as to why children under 12 months are excluded from many studies of these measures when changes in serological readouts for the very young may be most helpful in TBV trials. Drakeley replied that the measure is relevant to the whole population rather than a specific age group but measuring serological changes over time in children may be the most sensitive measure of changes in transmission.

**Modelling impact of anti-malarial interventions**

The second session provided an overview of current leading approaches to mathematical modelling of malaria parasite control measures and transmission. Three modelling groups were invited to communicate with each other and decide how to present data on the following: What questions related to impact of interventions are best answered by modelling? What do modellers see as the outcomes of interest for impact on transmission? What field data is necessary to make model predictions more robust?
Christinah Chiyaka, from the University of Florida, introduced this topic by outlining how models can be useful to measure changes in human, vector or parasite endemicity, to define intervention coverage levels and timelines, to predict outcomes, to assess risk factors and, to determine suitability for malaria elimination. These changes are estimated through entomological, epidemiological, and serological measures of transmission. Maps of \( P \) falciparum risk have been developed using some of these measures of malaria transmission. However, challenges to the quantitative validity of using entomological metrics for control were raised by studies that used different measures of transmission and obtained very different estimates of the basic reproductive number \( R_0 \), which describes reduction in transmission required to control malaria.

The enormous discrepancy between estimates of \( R_0 \) made by the number of infections per person per unit time (FOI) and average number of infectious bites per person per unit time (EIR) led to a recent study by D. Smith et al. (2010), where malaria data was assembled and analyzed to look for patterns that exist across the spectrum of transmission intensity. The classical Ross-Macdonald model assumes that there is a linear relationship between (FOI) and (EIR). The number of infections per infectious bite (FOI/EIR) describes the efficiency of transmission. However, in high transmission settings, malaria parasite transmission has been shown to be extremely inefficient. Smith et al. examined the relationship between daily or annual FOI and daily or annual EIR using assembled data from available studies. Instead of a straight line relationship, as the EIR increases, the FOI levels off (i.e. transmission efficiency decreases). There are many possible reasons for lower transmission efficiency at higher EIR, including lower or no parasite transmission from infectious mosquito to a human during blood meals, sporozoites may fail to advance to blood stages, immunity to blood stages could rapidly clear primary merozoites and suppress parasite densities below detectable levels, and heterogeneous biting, in which a few people receive most of the infectious mosquito bites. They found that a model that includes heterogeneous biting explains the non-linear relationship between FOI and EIR, in particular if 20% of the population receives 80% of the bites.

The discrepancy between the FOI and EIR, however, is particularly relevant for estimating transmission dynamics of malaria and control because measures of malaria transmission are used to stratify risk, plan for control, and evaluate responses to control.

Tom Smith, Swiss Tropical and Public Health Institute (Swiss TPH), reflected on the wide variety of uses of models, noting that predicting outcomes of interventions is only one of these. Sexual stage/mosquito antigen-transmission blocking vaccines might interrupt malaria parasite transmission in low transmission areas, but might also be included in integrated malaria parasite control programs, including in combination with other vaccines to protect them against selection of resistant variants. TBV are unlikely to be very effective in controlling malaria-associated morbidity and mortality.

Efficacy of TBV can be measured by the proportionate reduction in probability that a mosquito acquires infection at any given feed. It is agreed between the three modeling groups that this is a key outcome of interest from the modelers' perspective. In effect it is this parameter which is varied with different interventions to model impact on
transmission. Whilst it is of particular importance to modeling transmission impact, little field data is available of the effect of malaria interventions on this parameter. This is one key area where more field data may improve modeling predictions. Vaccine efficacy and population coverage are both relevant for TBV so the overall protective efficacy can be estimated by the product of these two factors. Smith argues that in the absence of intervention there is generally an endemic steady state where immunity keeps the effective reproduction number, $R_e$ at 1. $R_e$ would be reduced after vaccination, but eventually a new equilibrium would be reached as immunity adjusted. Prevalence would readjust to a new lower level at equilibrium, but there are many potential patterns that depend on decay of immunity. He referred to the classical Ross-Macdonald model, and noted that the effect of IRS was clear in reducing $R_e$ significantly from its equilibrium, but that this does not say anything about the transient dynamics of situations where transmission has been reduced or what to do to clear the residual infections.

The population size and their connectivity determine the effects of interventions on the interruption of transmission, in this case the ratio between infectiousness in vaccine groups and control. To model vaccine efficacy, mosquito infection rates are required as input parameters. These could be from wild-caught populations, in which case a measure of the survival time of the mosquitoes would be required or, if only a comparative measure is required, infection rates from direct or membrane feeding would be adequate. There is a problem, however, at low transmission intensities. Killeen et al(9) illustrate well how little data we have from areas with $EIR<10$, a critical situation in which vaccines may be assessed and later applied. As noted in Chiyaka’s talk, the relationship between the probability of a mosquito becoming infected and EIR plateaus with $EIR>10$; thus below this cut-off TBV could have substantial impact on transmission.

There is significant parameter and model uncertainty, which might be reduced by incorporation of features of several models. Vaccines need consideration in terms of transient dynamics and effects over time. The Swiss TPH models are stochastic and based on discrete time intervals of 1 or 5 days. Results from a variety of such models were shown for number and proportion of infections averted with several annual rounds of IRS followed by TBV. In general the effect was large for many years but by 20 years reverted to same or fewer infections averted by the combination, as with IRS alone. The models in the ensemble differed slightly, but having many models does not mean any of them is correct, (for example in HIV, predictions of demographic effects were not correct), but they can stimulate new approaches to problems. The presented multi-model ensemble indicated that TBV may not be better at interrupting transmission than other vaccines especially compared to pre-erythrocytic stages, and there is little benefit over other control methods, on morbidity and mortality, unless transmission is interrupted. In general the models indicate that the chance of elimination depends on coverage, initial transmission level, quality of surveillance, level of natural immunity, and human population size.

The final presenter in this section was Lucy Okell, Imperial College, and her stated aim was to “add realism to models for malaria (parasite) control and elimination”. Models should be able to deal with heterogeneity, human behaviour and different levels of
transmission. They should also aid decisions on which interventions should be combined, at what intensity and for how long. Transmission is heterogeneous with respect to intensity (EIR), seasonality, vector species and foci of transmission. Models can extrapolate potential interventions to different epidemiological settings; and tailor intervention packages to sites. Recent data show that control interventions are not isolated; countries where transmission is decreasing have had high bednet coverage, ACT introduced and also IRS in some settings concomitantly. Using data recently published(10) they examined the relationships between prevalence and EIR. The results predict outcomes for 6 sites with different EIRs and different interventions: MSAT (mass screening and treatment); LLINs; IRS; MSAT +IRS. Results were expressed as prevalence and proportional reduction in prevalence and varied greatly by site. In addition to interventions, the model also investigated human behavior such as bednet use and decay of efficacy (net durability issues) as well as heterogeneity in use of interventions in the same or different households. The model available on the Malaria Tools website allows users to input their own parameters. As there are limited field datasets available for parameterization of models, Okell emphasized the need for repeated GARKI type projects to generate more datasets to test models.

Okell then described results from a spatially-explicit, climate-driven, individual-based model exploring the relationship between the effect of interventions, in different EIR settings, on P. falciparum prevalence rates in 2-10 yr olds over Africa in a 20 year period, accounting for distribution of humans and vectors. In the version shown, LLIN were replaced every 5 years; IRS applied every 3 years, with 80% coverage and staggered timing.

The data needs for improved modeling were listed by Okell as:
- Vector species densities and relationship with EIR
- Better understanding of the pre-intervention relationship between transmission and climate, intervention use and socio-economic factors such as housing.
- The influence of super-infection on duration of infection and infectivity
- Infectious reservoir and age structure in lower transmission settings
- Rate of acquisition of immunity at different transmission intensity and rate of loss of immunity when transmission is reduced.

A key to achieving sustained control leading to elimination is to measure the infectious reservoir. Key questions relate to age, EIR, human infectiousness, immunity and seasonality in order to determine who and when to vaccinate with a TBV.

**Recent field data on human infectious reservoirs**

Andre Lin Ouedraogo described the human infectious reservoir in two different transmission settings in Burkino Faso before introduction of LLIN and ACT. They determined rates of gametocyte infection, by microscopy, and by quantitative nucleic acid sequence based amplification (QT-NASBA), and assessed infectiousness by DMFA. Significantly higher gametocyte prevalence rates were found with the QT-NASBA in both children and adults and the highest densities of gametocytes were in children (aged
1-14 years) but, as described previously, there was no direct correlation between gametocyte density and infectiousness to mosquitoes. Some individuals with high density gametocytaemias were non-infectious, and sub-microscopic gametocyte levels accounted for close to 50% of transmission. In the settings studied, more than 80% of transmitters would be covered by an intervention targeting up to age 14, and well over 90% by including those up to age 29, confirming the impression that targeting young children alone through vaccination is not a good strategy for reducing malaria parasite transmission. Interestingly, infectiousness was highest in this age group at the start of the transmission season, and then fell progressively, suggesting that transmission-reducing antibody titres had waned between transmission seasons. This talk also highlighted the remarkable paucity of good field data on human infectious reservoirs and that age patterns for infectiousness were likely to vary depending on intensity of transmission.

Assessment of SMFA for possible qualification

Carole Long summarized progress towards qualification of the standard membrane feeding assay (SMFA) which is the pivotal method of measuring TBV efficacy but has poor reproducibility. Using purified anti-Pfs 25 antibody, she and colleagues showed that transmission blocking activity is a function of antibody concentration but the precise IC$_{50}$ varies significantly between species(11). Furthermore, all show low reproducibility at low concentrations of antibody. As a first step towards qualification, the concentration of an anti-Pfs25 monoclonal antibody giving a consistently high inhibition of oocyst density was established, and this would be used as a comparator for all feeds. A more difficult second step is to establish whether a middle range of monoclonal antibody concentration could be qualified in relation to use of SMFA for pre-clinical studies and bridging to field studies. It was suggested that the assays could be quite variable, and that the output of SMFA should be a control/test ratio within in each experiment. A suggested application of a qualified SMFA was to compare antibodies to different sexual stage and mosquito stage antigens in preclinical animal studies.

Update on Gates Foundation and MVI funded TBV development

Progress in the development of candidate vaccines was reported. Janice Culpepper described the vector-based production of target antigens in tobacco plants by Fraunhofer CMB. The advantages of this vaccine technology are low cost and high yield. Full length forms of Pfs25, both glycosylated and non-glycosylated and with and without lichenase, were used for immunization. Pure, soluble, monomeric antigens induced antibodies with high transmission-blocking activity by SMFA. A major fragment of Pfs230 has also been similarly expressed and induced good antibody titres after vaccination in the presence of adjuvant and had high TB efficacy. A positive surface immune-fluorescence assay (SIFA) was a pre-requisite for activity by SMFA.

Ashley Birkett described PATH Malaria Vaccine Initiative (MVI) support for TBV and PEV focusing on transmission reduction. A draft target product profile (TPP) has been developed which includes both *P.falciparum* and *P.vivax*, and sets a high efficacy level.
(85% reduction in the probability of mosquito infection for each mosquito-human interaction) for a Go/No-go decision to be made at the pre-clinical stage. Two programmes are being supported, one on a Pfs 48/45 construct and the other on a novel antigen known as APN1. An adjuvanted, full length, codon-harmonised recombinant Pfs48/45 protein induced significant levels of transmission blocking antibody in mice and non-human primates. Further developments will include assessment of efficacy against a field isolate and development of a \textit{P. vivax} orthologue. The relationship between protein conformation and induction of blocking antibodies remains a product development challenge, and a better understanding of the impact of Pfs48/45 polymorphisms on efficacy against field isolates is needed. The APN1 candidate is an adjuvanted recombinant form of a mosquito midgut molecule that is involved in ookinete invasion. Polyclonal antibody response induced reduced \textit{P. falciparum} infection and interestingly anti-APN1 antibodies also have some \textit{P. vivax} activity.

Colleen Wood then described a MVI/TBV workshop related in scope to the current meeting and highlighted a presentation by US FDA. Here it was stated that lack of a direct effect does not preclude specific decision by FDA, but further details will depend on individual submissions. It is important to note that a major regulatory agency confirms the possibility of accepting benefit demonstrated at the community level for licensure of a vaccine, albeit with provisos. A further important contribution was the presentation by Christine Grady which explained how in the framework of the ethics of vaccine research, TBV trials could be considered ethical in principle. Grady outlined several other precedents for clinical research where trial subjects gain no direct medical benefit from participation in research. Working groups have been formed by MVI on the following areas: Clinical development, regulatory and policy issues; Assays and correlates; Baseline data & optimum transmission measures; Product design; Ethics and communication; Scenarios and implementation.

**Discussion & Recommendations on Key Issues**

Given the focus on reducing malaria parasite transmission, how can new anti-malarial interventions be evaluated from the perspective of transmission reduction? Outside the malaria field, it is often the transmission or indirect public health benefits of vaccination that are of equivalent or greater value than the direct effects. However vaccines are almost always licensed on the basis of direct benefits for the individual vaccinee, as measured by an effect on infection incidence, morbidity, or, if there is a clinically validated surrogate, immunogenicity. Often not well appreciated and/or not broadly communicated are the underlying major indirect benefits for vaccination; examples include rubella vaccination of toddlers to provide maternal protection against rubella infection during pregnancy, pertussis vaccination to protect newborns who are unvaccinated but at highest risk of severe sequelae(12), influenza vaccination of children to protect at-risk populations(13) etc. Oftentimes these transmission-related benefits are documented as part of post-introduction surveillance, for example for pneumococcal(14), meningococcal(15) and more recently rotavirus vaccines(16).
A breakout session at the meeting entered into a detailed discussion of the choice of endpoints for clinical trials of vaccines aiming to reducing transmission.

1. Baseline data to facilitate Phase 3 trial design for a malaria TBV
Agreement was reached on important aspects of design of a pivotal Phase 3 trial of a transmission-reducing intervention. A first distinction must be made between interventions with anticipated direct and possible indirect effects (such as a PEV), and interventions only anticipated to have indirect effects (the traditional TBV). If any component of the vaccine is considered to have only transmission blocking activity, its efficacy would need to be determined separately.

In some senses, designs of trials to measure indirect effects alone are simpler. Envisioning a cluster randomized trial whose primary objective is estimation of indirect effects, the group considered the three categories of measurement, namely incidence of infection or clinical cases (epidemiological measures), entomological measures and assay-based measures such as serology and nucleic acid tests. It was agreed that the primary endpoint for the pivotal Phase 3 trial will be incidence of infection, though the assay used to detect new incident infection remains to be agreed, and may or may not be traditional microscopy. A phase 3 trial will be such a major undertaking that it will be important to take the opportunity to collect data on the other categories of endpoints. A measure of EIR will be a secondary endpoint and one or more serological or nucleic acid assays should be included as a tertiary measure. In the course of doing such a study it would be very helpful to gain confidence in the use of serological and nucleic acid assays for use in post-introduction surveillance-related operational research.

The age groups to be immunized in such Phase 3 trials will depend on the infectious reservoir at the trial site; based on data currently available it seems highly likely that children from the age of 12 months or so up to adolescents will need to be included. Young adults may also need to be included and an argument could be made for immunizing the entire population, except for high risk groups such as pregnant women and those with evidence of immunocompromise. Modelling may help to decide groups for immunisation. The duration of follow-up will be 24 months or more to allow for temporal variation in transmission.

2. A priority need for transmission-related field epidemiology research
Further work is necessary to characterise the field trial sites where such studies could be performed. The discussion around which transmission settings to choose for such a trial highlighted the fact that epidemiological measures such as incident infection are less likely to show a reduction through TBV vaccination in high transmission settings but there may be concerns about power to detect an effect for reduction of incidence of infection if transmission is too low. Thus the transmission setting will need to be carefully chosen with baseline data from the proposed site. The meeting confirmed the remarkable paucity of data on transmission-related malaria epidemiology in field sites; there is a substantial priority R&D agenda for baseline work to determine human infectious reservoirs, characterise heterogeneity of malaria parasite infection risk (both spatially and temporally at potential trial sites), better understand naturally acquired
transmission-blocking immunity, and link DFA, DMFA, SMFA and IgG titres to candidate sexual stage antigens. The confirmation that microscopy for gametocytes misses many infectious individuals within communities confirms the need to compare any proposed assay to identify transmitters with a gold standard assay of infectivity. If DFA is considered unacceptable, DMFA may need to be used for this comparison. In any case the paucity of infrastructure to assess infectiousness in endemic countries could be addressed through research funding. This transmission-related epidemiology will be necessary to identify the clusters to be used in the Phase 3 trial.

3. Immunogenicity and functional immunoassays for evaluation of sexual stage and mosquito antigen candidate vaccines

The meeting confirmed two points: first, the major advantage of the existence of functional assays of infectivity for TBV and secondly the importance of developing an assay with acceptable precision. If a functional assay can be qualified and convincing links can be shown between assay readouts and clinical endpoints reflective of interruption of malaria parasite transmission in a population, discussions with regulatory authorities may enable certain accelerations in the regulatory pathways involved. If on the other hand assay characteristics are judged to be unacceptably variable or with insufficient links to clinical benefit, regulators are likely to require a pre-licensure Phase 3 trial to demonstrate transmission reduction. As discussed above, there are major challenges in the design of such trials, which are also likely to take significant time and resources to complete. Thus a key branching point for the field of TBV development will be the success or failure of functional assay development. An alternative way forward would be to demonstrate a sufficient link between non-functional immunoassays such as IgG ELISA and clinical endpoints bridging through intermediate functional assays.

4. The role of Phase 1b-2b vaccine studies in endemic countries for TBV

Vaccine researchers presented data to indicate that safety and immunogenicity and a degree of functional activity will be required for progression from Phase 1a to endemic countries. However there is further need for focused discussions by a working group on the possible benefit of small Phase 1-2 studies in endemic countries and possibility of demonstrating proof of principle of TBVs in such settings. In particular, the possibility of demonstrating a reduction in the infectivity of vaccinated humans to mosquitoes in phase 2b studies remains less well developed in terms of study design and analysis methods. If such a trial can be designed to prove that the vaccine has a biological effect on infectivity, this is likely to generate resources to conduct the pivotal Phase 3 trial, and so may be a driver of progression of the TBV field. Some stakeholders also see agreement of one or more target product profiles as important drivers for TBV vaccines. Iterative cycles of phase 1-2 trials in adults in endemic countries may be needed to refine the information that can be obtained from such trials.

5. The role of modelling in evaluation of TBVs

One agreed interaction between field research and modellers is the constant need to strengthen model parameterization and refine assumptions based on field data. There is a valuable dialog to be had between modellers and field researchers on the types of
epidemiological data which will strengthen existing models. Current models are forced to rely on a few detailed datasets such as the Garki project where a range of outcome measures are available before, during and after the course of a field intervention. Field researchers are encouraged to work with modelling groups to improve modelling outputs in an iterative fashion. The concept of sensitivity analysis is important and both researchers and policymakers need to understand the key assumptions and parameters for existing models, and the relative sensitivity of predictions to small changes in inputs. If the TBV field is one in which clinical trial efficacy data is comparatively difficult to obtain, then modelling may have a greater role earlier in the development process, and this would be facilitated if concerns about robustness of predictions are addressed. At present, opinions vary widely about the usefulness of, and applications for, mathematical models in predicting the public health value of TBVs. The approach followed at this meeting where leading mathematical modelling groups work together to answer questions is encouraged and could be further developed.

6. Towards standardization of measures of transmission
Drakeley, Sauerwein, Long and Ouedraogo outlined the complex assay environment for possible measures. Behind present assays and diagnostic methods used routinely as part of malaria control programmes, there are several other assays such as LAMP, PCR, QT-NASBA, luminex, microarray and IgG ELISA for various antigenic targets. Each of these has pros and cons for measurement of exposure to, or presence of, asexual and sexual stage parasites. It is clear that some of these assays could have an important role in improving detection of the infectious reservoir in pre-elimination and elimination scenarios. There may also be a role for monitoring of impact of control measures. The meeting highlighted the poor performance of asexual and gametocyte microscopy for detecting the infectious reservoir and while an assay of infectivity such as DFA and DMFA remains most valuable for this purpose, nucleic acid based assays that detect ultra-low density gametocytes such as QT-NASBA may be useful in detecting humans infectious to mosquitoes. Further field data on the performance of these assays from multiple transmission settings and in the context of introduction of anti-malarial interventions would be valuable.

7. P. vivax and transmission
The specific issues related to P. vivax R&D have been summarized in other consultations, and these include major differences in vector ecology and behaviour, P. vivax's comparative intransigence to many forms of malaria control, and the complexity introduced to trial design by the presence of the hypnozoite stage of the life-cycle. If expected gains are made with P. falciparum control, P. vivax will have higher priority for R&D.

Conclusion
This meeting has updated the previous WHO document on transmission-blocking vaccines "an ideal public health good" and endorses the need for working groups on key issues outlined above. Five years from now there should be information on the performance of some of the vaccines progressing through pre-clinical R&D in 2011 and it will be essential that more information is available about the relative utility of
functional assays of infectivity for decision-making in TBV vaccine development. At that time it would be highly desirable if further data were available from transmission-related field epidemiological research in several settings to allow design of field trials of TBV with confidence that meaningful transmission reductions will be detectable in a pivotal Phase 3 trial.

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Participants MALVAC Meeting 15-16 November 2010

- Dr John W Barnwell, Division of Parasitic Diseases, Centers for Disease Control and Prevention, Chamblee, 30341, Georgia, USA
- Dr Teun Bousema, London School of Hygiene and Tropical Medicine, London, WC1B 3DP, UK
- Dr Sophie Biernaux, Malaria Franchise Head, GlaxoSmithKline Biologicals, Rixensart, 1330, Belgium
- Dr Fred Binka, School of Public Health, College of Health Sciences, University of Ghana, P.O. Box LG13, Legon, Ghana
- Dr Ashley Birkett, Director, Pre- and Early-Clinical Research and Development, PATH Malaria Vaccine Initiative, Bethesda, MD 20817, USA
- Dr Carla Botting, Director Commercial Affairs and Product Development, PATH Malaria Vaccine Initiative, Bethesda, MD 20817, USA
- Professor Graham Brown, Meeting Chair, Director, Nossal Institute for Global Health, University of Melbourne, Level 5, Alan Gilbert Building, Victoria 3010, Australia, Melbourne, Australia
- Ms Terrell Carter, Program Officer, PATH Malaria Vaccine Initiative, Bethesda, MD 20817 USA
- Dr Roma Chilengi, Wellcome Trust Research Laboratories, PO Box 230, Bofa Road, Kilifi, Kenya
- Dr Christinah Chiyaka, Department of Zoology, University of Florida, Gainesville, Florida, USA
- Dr Joe Cohen, GlaxoSmithKline Biologicals, Rixensart, 1330, Belgium
- Professor Marc Coosemans, Head, Unit of Medical Entomology, Department of Parasitology, Institute of Tropical Medicine, Antwerp, Belgium
- Dr Janice Culpepper, Senior Program Officer, Infectious Diseases, Global Health, The Bill and Melinda Gates Foundation, Seattle, WA 98107-5136, USA
- Professor Ogobara Doumbo, Director, Malaria Research and Training Center, University of Bamako, Mali
- Dr Chris Drakeley, London School of Hygiene and Tropical Medicine, London, WC1B 3DP, UK
- Dr Patrick Duffy, Chief, Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Disease, Rockville, MD, USA
- Dr Adam Fimbo, Tanzania Food and Drug Authority, Dar-es-Salaam, United Republic of Tanzania
- Dr Blaise Genton, Policlinique Médicale Universitaire, 1011 Lausanne, Swiss Tropical Institute, 4002 Basel, Switzerland.
- Dr Louis Clément Gouagna, Institut de Recherche pour le Développement, Montpellier, France
- Dr Patricia Graves, Malaria Control Program, Carter Center, One Copenhill, 453 Freedom Parkway, Atlanta, GA 30307, USA
- Dr Stephen L Hoffman, Chief Executive and Scientific Officer, Sanaria Inc, 9800 Medical Center Drive, Suite A209, Rockville, 20850 MD, USA
− Dr Egeruan Babatunde Imoukhuede, Director, Clinical and Regulatory Affairs, European Vaccine Initiative, University of Heidelberg, Heidelberg, Germany
− Dr T. Jacob John, Formerly Professor & Head, Department of Clinical Virology, Christian Medical College Hospital, India
− Dr Robert Johnson, Director, Office of Regulatory Affairs, Division of Microbiology and Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, MD 20892, USA
− Dr Eric Karikari-Boateng, Acting Head Laboratory Services, Food and Drugs Board, Accra, Ghana
− Dr David Kaslow, Vice-President Vaccines & Infectious Diseases, Merck Research Laboratories, New Jersey, USA
− Dr Amanda Leach, Clinical Development, GlaxoSmithKline Biologicals, Rixensart, Belgium
− Dr Ralph Leblanc, Center for Biologics Evaluation and Research, Food and Drug Administration, Rockville, MD 20852, , United States of America
− Dr Didier Leboullex, Associate Director, PATH Malaria Vaccine Initiative, Bethesda, MD, 20817, USA
− Dr Odile Leroy, Director, European Vaccine Initiative, University of Heidelberg, Heidelberg, Germany
− Professor Steve Lindsay, Chair of Public Health Entomology, Room 410, Disease Control & Vector Biology Unit, Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine,Keppel Street, London WC1E 7HT, UK
− Dr Carole A. Long, Chief, Malaria Immunology Section, Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Disease, Rockville, MD, USA
− Dr Christian Loucq, Director, PATH Malaria Vaccine Initiative, Bethesda, MD 20817, USA
− Dr Michael Makanga, Capacity Development Manager, EDCTP, Francie van Zijl Drive, Parow, P.O. Box 19070,Tygerberg, 7505, South Africa
− Dr Kamini Mendis, Independent Consultant, Colombo, Sri Lanka
− Dr Paul Milligan, London School of Hygiene and Tropical Medicine, London, WC1E 7HT, UK
− Ms Jessica Milman, Senior Program Officer, Infectious Diseases, Global Health Program, The Bill and Melinda Gates Foundation, Seattle, 98102 WA, USA
− Professor Malcom Molyneux, University of Malawi, Blantyre, Malawi
− Dr Patricia Njuguna, Wellcome Trust Research Laboratories, PO Box 230, Bofa Road, Kilifi, Kenya
− Dr Julia Nunes, Program Officer, PATH Malaria Vaccine Initiative, Bethesda, MD 20817, USA
− Dr Lucy Okell, Department of Infectious Disease Epidemiology, Imperial College, London, UK
− Dr André Lin Ouédraogo, Department of Biomedical Sciences, Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso,
− Dr Jayesh Pandit, Head, Dept. of Pharmacovigilance, Ministry of Medical Services, Kenya
− Dr Maria Grazia Pau, Program Director Vaccines, Crucell, Archimedesweg 4-6, P.O.Box 2048, 2301 CA Leiden, The Netherlands
− Dr Inmaculada Penas-Jimenez, European Commission, DG RTD, Health Research, Rue du Champs de Mars, 21, B-1050 Bruxelles, Belgium
− Dr Margaret Pinder, Medical Research Council Laboratories, Atlantic Road, Fajara, PO Box 273, Banjul, Gambia
− Dr Claudio Ribeiro, Laboratório de Pesquisas em Malária, Instituto Oswaldo Cruz, Fiocruz
− Pavilhão Leonidas Deane - 5º andar, Av. Brasil 4365 CEP 21045-900, Rio de Janeiro, R.J, Brazil
− Dr Steve Rosenthal, Division of Microbiology & Infectious Diseases, NIH/NIAID, Bethesda, 20892 MD, USA
− Dr Edith Roset-Bahmanyar, Epidemiologist, GlaxoSmithKline Biologicals, Rixensart, Belgium
− Dr Robert Sauerwein, Department of Medical Microbiology/Parasitology (MMB 574), University Medical Center St. Radboud, 6500 HB Nijmegen, Netherlands
− Dr Robert Sinden, Imperial College London, South Kensington Campus, London SW7 2AZ, UK
− Professor Peter Smith, Department of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London, WC1E 7HT UK
− Professor Thomas Smith, Swiss Tropical and Public Health Institute, Basel, Switzerland
− Dr Aaron Sosola, Medicines and Poisons Board, Ministry of Health, Lilongwe, Malawi
− Professor Geoff Targett, Emeritus Professor of Immunology of Parasitic Diseases, London School of Hygiene and Tropical Medicine, Room 406, Keppel Street, London WC1E 7HT, UK
− Dr Mahamadou A. Thera, Department of Epidemiology of Parasitic Diseases, University of Bamako, Bamako, 1805 BP, Mali
− Dr Aissatou Toure-Balde, Laboratoire d'ImmunoParasitologie, Institut Pasteur, Dakar, Senegal
− Dr Marita Troye-Blomberg, Dept of Immunology, University of Stockholm, The Wenner Gren Institute, Arrenius Labs F5, S-10691 Stockholm, Sweden
− Dr Johan Vekemans, Pediatric Malaria Vaccine Development, GlaxoSmithKline Biologicals, Rixensart, Belgium
− Dr Eleonora G. Wijnans, Clinical Assessor, Medicines Evaluation Board, Postbus 16229, 2500 BE Den Haag, The Netherlands
− Dr Colleen Woods, Program Officer, PATH Malaria Vaccine Initiative, Bethesda, MD, 20817, USA

WHO Secretariat
− Prof Bartholomew Dicky Akanmori, Research and Development New Vaccines Officer, Immunization and Vaccine Development Programme, WHO, AFRO Region, Congo-Brazzaville
− Dr Maria Baca-Estrada, Scientist, Quality, Safety and Standards, Initiative for Vaccine Research, WHO, Geneva 27, 1211, Switzerland
– Dr Georges Ki-Zerbo, Regional Adviser, Malaria, WHO, AFRO Region, Congo-Brazzaville
– Dr Vasee Moorthy, Technical Officer, Initiative for Vaccine Research, WHO, Geneva 27, 1211, Switzerland
– Dr Robert Newman, Director, Global Malaria Programme, WHO, Geneva 27, 1211, Switzerland
### Box 1: Terminology related to malaria parasite transmission and vaccines

Until recently the acronym TBV (transmission-blocking vaccines) was commonly used in the malaria vaccine R&D community to refer to vaccine constructs based on the sexual and sporogonic stages of *Plasmodium* parasites. These were envisaged as vaccines that would prevent transmission from humans to mosquitoes by targeting the stages of the parasite responsible for this step of the life-cycle. However it is recognised that a sufficiently effective vaccine targeting any stage of the life-cycle could theoretically reduce malaria parasite transmission. Thus more recently the term "Vaccines that Interrupt Malaria (parasite) Transmission" (VIMT) was suggested by the vaccines stream of the Malaria eradication R&D agenda-setting process. The concept of VIMT is that whatever the vaccine, the outcome of interest for elimination and eradication is reduction of malaria parasite transmission. During the MalERA process it was highlighted that further work was necessary to agree upon appropriate clinical and regulatory strategies to document efficacy in terms of reduced transmission. In this document we use PEV (pre-erythrocytic vaccine), BSV (blood-stage vaccine) and TBV to refer to life-cycle stage-specific vaccines, and VIMT to refer to vaccines that target any life-cycle stage and whose public health value is conceived in terms of its transmission reduction effect. It is easy to conceive of a highly efficacious PEV that, by preventing almost all blood-stage infections, would prevent disease, death and malaria parasite transmission. It is also conceivable that a highly efficacious BSV may reduce transmission by an impact on gametocytaemia. In addition to candidate vaccines that specifically target sexual (i.e., gametocyte and gamete) and sporogonic (i.e., zygote and post-zygote) stage parasites, mosquito antigen vaccines that target mosquito midgut and block malaria parasite transmission without inducing an immune responses to any parasitic target or impacting the mortality or fecundity of the mosquito are also contained within the term TBV.
Box 2: Malaria Parasite Transmission Epidemiology
Kamini Mendis reviewed the transmission framework for malaria parasite epidemiology and conceptualized the ways that malaria parasite interventions can reduce transmission. The idealized unit of measurement of the transmission of malaria parasites is the reproduction number \( R \) which is the number of new malaria parasite infections generated by a single infected individual. This is an expression of the efficiency of the mosquito vector (vectorial capacity) \( C \) and the magnitude of the infective parasite pool in humans (denoted by the daily rate of loss of human infectivity) \( r \) as follows:

\[
R = \frac{b \cdot C}{r}
\]

where \( b \) is the proportion of mosquito bites which are actually infective. For malaria and malaria parasites to be eliminated, the reproduction number must be held below 1 until the pool of parasites has been removed. The parameters that determine vectorial capacity \( C \) are the density of mosquitoes \( m \), their feeding frequency on humans \( a \), their daily survival rate \( p \) and the duration of the parasite’s development cycle in the mosquito (sporogonic cycle) \( n \) and is expressed as,

\[
C = ma^2 p^n \cdot \frac{1}{-\log_e p}
\]

Vectorial capacity is extremely sensitive to changes in the daily survival of the mosquito, e.g., a 20% reduction in the mosquito’s daily survival rate could result in a 98% reduction in vectorial capacity, whereas, under the same circumstances, a similar reduction in the vector densities will result in no more than a 20% reduction in vectorial capacity, respectively. The nature and intensity of malaria parasite transmission (and the susceptibility of malaria parasites to interventions) depend largely, therefore, on the bionomics of the mosquito vector prevalent in the area.

The relation between the prevalence of malaria parasite infections, inoculation rate, vectorial capacity and reproduction number is represented in Figure 1. Measures of prevalence of infection are dependent on the threshold of detection of the assay (see figure 2). The entomological inoculation rate (EIR) is the number of infectious mosquito bites received per person per unit time. In situations with annual EIR below about 10, the prevalence of malaria parasite infections is almost directly proportional to EIR, and, conversely, malaria parasite control measures lead to an almost proportionate reduction in both the prevalence of malaria parasite infections and the incidence of disease. In situations in which this range of inoculation prevails, malaria parasite transmission tends to be unstable and is considered to be of low-to-moderate intensity. At annual EIR of above 10 or so, people receive many infectious bites, which leads to overlapping infections, a state referred to as ‘superinfection’. In this case, a reduction in inoculation rates by malaria parasite control methods will reduce the incidence of disease but not the malaria parasite prevalence, until entomological inoculation rates are lowered to below 10 or so.
In situations where EIR is higher, malaria parasite transmission intensity is considered high and tends to be stable, although entomological inoculation rates below 10 are found in some areas of stable transmission. Malaria parasite transmission is anything but homogeneous. As there is heterogeneity in both the distribution of malaria parasite inoculations in a population and the susceptibility of humans to infection, a small proportion of people tend to receive a large proportion of incident infections. This heterogeneity in human–mosquito contact and human susceptibility to malaria parasite infections is an important factor in increasing the tenacity of transmission.
Figure 1: The relationship between vectorial capacity, basic reproduction rate, entomological inoculation rate, and prevalence of parasitaemia

Based on Dietz et al., (1974) and adapted from Wernsdorfer & McGregor (1989)

Source: Global malaria control and elimination, WHO, 2008
Figure 2. Relationship between inoculation rates and parasite prevalence by microscopy or PCR
The number of new cases = \( x \times (R_0)^y \)

- **Mass treatment & PEV**
- **Number of cases at time 0**
- **Vector Control**
  - \( \sim \) Vectorial capacity
- **Early treatment of cases**
  - \( \sim \) duration of infection
- **Number of cycles of transmission**
- **Gametocytocidal drugs & VIMT**
  - \( \sim \) proportion mosquitoes infected
Figure 4: Sexual-stage antibody responses to *P falciparum* in endemic populations

J.T. Bousema, C.J. Drakeley & R.W. Sauerwein