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Criteria for Identifying and Evaluating Candidate Sites for Open-Field Trials of Genetically Engineered Mosquitoes

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Abstract

Recent laboratory successes in the development of genetically engineered mosquitoes for controlling pathogen transmission have fostered the need for standardized procedures for advancing the technical achievements to practical tools. It is incumbent in many cases for the same scientists doing the in-laboratory discovery research to also take on the initial challenges of developing the pathway that will move the technologies to the field. One of these challenges is having a set of criteria for selecting collaborators and sites for efficacy and safety field trials that combine rigorous science with good ethical and legal practices. Specific site-selection criteria were developed in four categories—Scientific, Regulatory, Community Engagement, and Resources—in anticipation of open-field releases of a transgenic mosquito strain designed to suppress populations of the dengue vector mosquito, *Aedes aegypti*. The criteria are derived from previous published material, discussions, and personal experiences with the expectation of providing guidance to laboratory scientists for addressing the conceptual and operational considerations for identifying partner researchers and countries with whom to collaborate. These criteria are not intended to be prescriptive nor can they be applied to every circumstance where genetic approaches are proposed for deployment. However, we encourage those involved in the discovery phase of research to consider each criterion during project planning activities, and where appropriate, incorporate them into a ''go/no-go'' decision-making process for further development and testing of the technologies.

Key Words: Genetic control—Open-release—Transgenic mosquito—Field trial—Community engagement—GMO regulation.

Introduction

Much of the current available vector control technology dates back a quarter of a century or more. In that time, many vector-borne pathogens have increased in incidence, prevalence, and geographic distribution (Kilpatrick and Randolf 2012). There is an urgent need for options and tools to augment current community-, biocontrol-, and insecticide-based strategies. This need has prompted efforts to develop novel vector-control strategies that are safe, efficient, and cost-effective (Cohen 2005, Hemingway et al. 2006, World Health Organization/Tropical Disease Research 2010). New and existing vector-control interventions require evidence-based assessments to optimize operational control policies and practices (Chanda et al. 2011). Progressive testing is essential as new tools move from benchtop discovery to practical use in the field to satisfy safety and performance requirements of stakeholders, including developers, collaborators, scientific oversight committees, regulators, and, importantly, communities in which the technologies will be deployed (World Health Organization/Tropical Disease Research 2010).

Genetic strategies are based on the premise that vector-targeted disruption of transmission of a pathogen results in reduced morbidity and mortality in humans. Genetic-based strategies can have the goal to eliminate or reduce mosquito densities below transmission thresholds through population suppression or to establish mosquito populations that are refractory to the pathogen (population replacement/modification) (Milani 1967, Curtis 1968, Collins and James 1996, James

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2000, James et al. 2006). These strategies are anticipated to work synergistically with current and other proposed disease management programs. Notably, several strategies are argued to become increasingly more efficacious and cost-effective as the size of the target wild mosquito population diminishes. However, significant challenges exist because genetics-based approaches cannot take advantage of the well-established development and delivery pathways of new insecticides, drugs, and vaccines. Trial designs, regulatory requirements, efficacy, and safety end points are well established and standardized for more conventional tools, but they must be adapted or developed *de novo* for the new technologies.

Progress in genetic-based tools

Significant advancements have been made in the discovery of genetic-based products to control vector-borne diseases (some examples for addressing transmission of dengue viruses are included in Franz et al. 2006, Phuc et al. 2007, Nawtaisong et al. 2009, Fu et al. 2010, Mathur et al. 2010, Labbé et al. 2012). Efforts to develop a population-suppression strain based on a refinement of a female-killing strategy (Black et al. 2011) yielded transgenic *Aedes aegypti* carrying a conditional dominant gene that rendered females unable to fly (Fu et al. 2010). This technology also was demonstrated recently in *Ae. albopictus* and a malaria vector mosquito, *Anopheles stephensi* (Labbé et al. 2012, Marinotti et al. 2013). Female-specific activity for the release of insects carrying a dominant lethal gene (female-specific flightless [fsRIDL]) allows for genetic sexing and has additional logistical benefits associated with the manufacturing and delivery of the product (Thomas et al. 2000, Alphey et al. 2008). A proof-of-principle of this population suppression strategy was demonstrated in large laboratory cages containing genetically diverse target populations (Wise de Valdez et al. 2011). Release of nonsexed fsRIDL pupae into target populations at an approximately nine fsRIDL males to one wild-type male ratio eliminated those populations in 10–20 weeks. A recent trial of the same fsRIDL strain in large field cages revealed a mating competitiveness deficiency that necessitates refinements of the approach (Facchinelli et al. 2013). However, these combined data support the further evaluation of fsRIDL technology as part of overall population suppression control efforts.

A phased approach to testing

The fsRIDL technology was tested in both Phase I and Phase II (World Health Organization/Tropical Disease Research 2010) field-cage trials in anticipation of an open-release trial. The Phase II field-cage trials were conducted to evaluate influences such as exposure to a wide variety of indigenous microbial flora and fluctuations in temperature, humidity, wind, and light not found in laboratory conditions (Facchinelli et al. 2011, Facchinelli et al. 2013). Stakeholder assessments of risks and benefits guide the decision-making process that determines whether product testing occurs in contained-field trials (*i.e.*, large cages in an outdoor setting) or in confined-field trials (sites that offer geographical, environmental, or biological confinement) (World Health Organization/Tropical Disease Research 2010). Although field cages can approximate environmental influences, performance evaluations under truly natural conditions require open-field trials.

Site selection

Most, if not all, of the new technologies are coming out of laboratories located in developed countries. The intention of the scientists in these laboratories is to move the advances from their benches to the field. Therefore, an important milestone is the selection of a site that allows credible testing of the technology. This requires the laboratory scientists to evaluate and choose such a site.

Many factors need to be considered when selecting a potential field-trial site for testing genetic-control strategies (Knols and Bossin 2006, Lavery et al. 2008, Harris et al. 2011, Harris et al. 2012, Lacroix et al. 2012). Identifying and evaluating criteria for selecting a trial site is a process coupled with product performance measurements. The site-selection process must include the active participation of stakeholders and a thorough and rigorous evaluation of ethical, social, and cultural (ESC) considerations (Lavery et al. 2008, Lavery et al. 2010). The final decision for selecting a field site will be the shared responsibility of the developers/researchers and the trial-site collaborators. Relationships developed during the selection of the field site should influence the product development pathway and will play a critical role in the final evaluation of success or failure of product trials.

We describe here the evaluation criteria used in a collaborative effort to select field sites for open-field trials of an fsRIDL population suppression technology targeting the dengue vector mosquito, *Ae. aegypti*. Detailed checklists provided as Supplementary Tables (Supplementary Data are available at www.liebertonline/vbz/) evolved from experiences in identifying, selecting, and participating in contained and confined field trials and bring together guidelines developed previously (Knols and Bossin 2006, Benedict et al. 2008, Lavery et al. 2008, World Health Organization/Tropical Disease Research 2010). The checklists have been made more general to accommodate other vectors and disease pathogens. We emphasize that careful consideration of the concept behind each criterion must be made if the criteria are to be applied to vector species other than *Ae. aegypti*. These checklists were developed to address open-field trials of a noninvasive population suppression tool that is self-limiting by design. The checklists are neither exhaustive nor exclusive for fsRIDL but can be used as a resource in the development pathway for other types of genetic or novel strategies, acting as a starting point and a basis for further discussions. While they are amenable to other engineered product strategies, for example, anti-pathogen transgene introgression into target mosquito populations, additional consideration must be given to persistence and spread (*e.g.,* transboundary issues).

Four categories of criteria are considered to identify and then evaluate candidate field sites for evaluating efficacy end points—Scientific, Regulatory, Community Engagement, and Resources. The checklists include specific ''go/no-go'' criteria (Box 1) that would eliminate candidate sites from further consideration (*i.e.*, not just selecting the best from several candidates). Each category considers an array of specific questions and criteria that can be addressed quantitatively or qualitatively and be used to evaluate sites that have met the basic go/no-go criteria.

Although a number of requirements for open-release trial sites are generic, some issues depend on the specific trial design. Three types of trials are considered with scales,

Box 1. Minimum Selection Criteria of Potential Field Site for Testing Genetic Strategies to Control Mosquito Vector

- The target species must be present at the proposed site(s).
- There is no widespread opposition (public or institutional) to testing genetically engineered strategies.
- There is a credible regulatory structure in place to sanction all of the necessary research activities, including import/export, transport, and research.
- Resource commitments can be met by participants and participating institutions.
- There is a researcher or research team with expertise in vector biology that has local ties and is willing to be an enthusiastic collaborator.
- The location does not present unacceptable risks for project staff.

timelines, and settings adopted specifically for evaluating sterile-male releases as a strategies for control of *Ae. aegypti*.

1. ''Ranging Trial'' is a time-limited, minimal-scale (4–6 weeks of releases with 10,000–100,000 males released per week in a small geographic region) initial trial in which a known number of mosquitoes are released into a wild population (Harris et al. 2011, Lacroix et al. 2012). Postrelease transgenic-to-wild male and female mating ratios are estimated from adult traps and ovitraps, respectively. Data relating to the longevity and dispersal of released males also are obtained. Data from these experiments allow estimates of the size of the wild population, in-the-field mating competitiveness of transgenic males, and the release rate expected to suppress the target population. This small-scale trial does not have population suppression as an endpoint.
2. ''Suppression Trial'' also is a time-limited, small-scale (4–6 months of releases with $\sim$100,000 males per week) trial release (Harris et al. 2012). The end points are the same as with a Ranging Trial, with the major addition of also emphasizing target population suppression. The release program is intended to have a measurable impact on the numerical size of the wild mosquito population in the release area.
3. The ''Pilot-Scale Intervention Trial'' is a larger release, 12–24 months in duration covering one or more significant human settlements of $\geq$10,000 inhabitants. Release numbers are calibrated to the size of the geographic area and target mosquito population density. The end points are similar to the Suppression Trial, but depending on the location and trial design, epidemiological measures/end points also may be feasible. Measurements of the impact on epidemiology are expected to be complex and costly (James et al. 2011, Wolbers et al. 2012).

The trials for which the checklists were developed were designed to evaluate efficacy and obtain biosafety data (such as longevity and dispersal, species ratios in traps, etc.). However, many of the features of the criteria can be applied to all of the trial designs. The three trial types likely will be undertaken consecutively, although the availability of sufficient baseline data regarding the target mosquito population, and/or data from other sites, could support initiating the series with a Suppression Trial. If resources permit, use of more than one release area in a given trial would be desirable. Control sites are required and should include historical data where it exists. The size, number and relative distance of control sites will be a significant component of the trial design (James et al. 2011, Wolbers et al. 2012).

Results and Discussion

Scientific

Biological/technical criteria relate to characteristics of the specific proposed trial site that would potentially impact trial design and result validation, and attempt to account for additional confounding factors that would otherwise make the site unsuitable. The criteria are applicable to both experimental and control locations (Table S1).

Entomological data

Presence of target species. Genetic strategies are designed to impact wild populations of mosquito vectors through population suppression or population replacement. Therefore, testing efficacy and safety is more relevant when the target species is present at the trial site. This is an obvious requirement for testing efficacy, but also is important for evaluating safety criteria (risk assessment). Suggestions for conducting initial releases in areas that are devoid of the target species due to inhospitable conditions may seem appealing to some from a risk assessment perspective. However, these sites do not have the appropriate physical properties to support the insects and extrapolation from them to actual control regions is problematic. Moreover, if the site is to be used later to measure an impact on disease transmission, the target species should not only be present but should also represent the principal vector of transmission (see Presence of disease section, below).

The approximate abundance of both the target species and other relevant mosquito species in the area must be known (see Presence of other mosquito species section, below). If target mosquito population densities are high, local public health authorities may need to intervene with vector-control measures to prevent an outbreak of disease unrelated to the field trials. While the likelihood of the need for conventional vector-control should be taken into account during planning, use of alternate interventions (insecticides, source reduction) by public health authorities would not preclude a trial if care is taken to ensure similar intervention measures are used at both experimental and control sites (see Other vector-control activities, below). In contrast, low target vector population densities will result in low signal-to-noise ratios, making it difficult to measure the efficacy of the intervention, especially in areas that are prone to mosquito immigration.

The recruitment rate (mosquitoes produced/day) is the most relevant measure of the target population against which to compare release rates of transgenic insects. In principle, pupal surveys allow an estimate of this recruitment rate; however, they are difficult to conduct rigorously, and other adult or immature indices are used more commonly, even though they are related less directly to the recruitment rate.

Assessing mosquito population size is difficult. Most methodologies are based on indirect measures of juvenile life stages (eggs, larvae, or pupae) that generally correlate poorly with adult population numbers. Mark/Release/Recapture methods to estimate adult numbers directly are sensitive to the effects of the target mosquito population density, the absolute area surveyed, dispersion patterns of the marked mosquitoes, and the number and/or location of release and recapture sites (Valerio et al. 2012). Regardless of the approach, survey methods vary significantly from country to country. This is due partly to historical adoption and development of different systems by vector control units, but also reflects different social/cultural, economic, and ecological factors. For example, BG Sentinel traps for capturing adults are expensive and need a constant power supply (*e.g.*, line or heavy-duty battery), making their use impractical in many locales. Cultural factors may preclude other methods, such as aspiration and larval/pupal surveys, which require intrusive inspections with survey teams entering households. Comparisons among countries are difficult even when the same methods are used because environmental factors (housing type, population density, climate, etc.) affect the sensitivity of survey systems. Many survey methods also are dependent on operator skill, and subtle changes in methodology can have large effects on results. Having detailed potential problems, the following broad criteria can be used in conjunction with the practical knowledge of experienced vector control scientists in assessing population sizes to assess the merit of candidate sites.

The lower limit will be defined generally as the minimum population size that can be detected reliably by the monitoring system(s) in use, taking into account the stochastic fluctuation in even a relatively stable mosquito population. This determination is dependent on the monitoring intensity (number of traps, etc.), which in turn has practical and financial limitations.

The upper limit is defined as that at which additional methods for vector control are required to maintain good public health practices. If population levels (as measured by the local adopted monitoring systems) approach levels that would trigger intervention by local vector control agencies, the likelihood of such intervention should be incorporated into trial plans. Table 1 indicates some low and high values in various survey systems, although these should not be taken as definitive. All limits should be defined on a case-by-case basis and design approaches to gathering the useful data can be developed with local experts.

Table 1. Examples of Mosquito Density Metrics Used to Inform Control Efforts

Mosquito metric	*Lower limits*	*Upper limits*
Ovitrap index[a]	< 10%	> 30–50%
BG traps	?	~1 *Ae. aegypti*/trap per night
Pupae/person[b]	< 0.05	1–2 pupae/person

[a]The Ministry of Health in Malaysia recommends intervention in levels exceeding 10% (Tee et al. 1997: Vector Control Unit, Ministry of Health, Malaysia).

[b]Theoretical dengue epidemic transmission threshold ranges from 0.06–23.3, dependent on temperature and immunity in human population (Focks et al. 2007).

Presence of other mosquito species. fsRIDL is a species-specific intervention and therefore the presence of other mosquito species in the locale will not interfere directly with the trial. A number of considerations are associated with the presence of other species:

- If other species are attracted to the monitoring traps, additional efforts and resources are required to sort these out to be able to analyze *Ae. aegypti* specifically.
- It is possible that in some cases other species will provide a useful internal control.
- The presence of other, less efficient, dengue vectors requires specific consideration if epidemiological endpoints are desired.
- Potential impacts on nontarget species require careful analysis for regulators and local communities.

Human inhabitants. *Ae. aegypti* are anthropophilic thus necessitating evaluation of potential trial sites in or near human habitation centers. Moreover, in areas of endemic disease, large-scale trials and intervention programs will be designed ultimately to have epidemiological end points intended to protect human populations. Therefore, efficacy will have to be tested on target mosquito populations under peridomestic conditions where disease transmission could occur. For example, efforts to control urban dengue require targeting mosquitoes in densely populated areas.

Human populations vary in terms of demographics (*i.e.*, information about race, sex, age, disabilities, mobility, education, employment, etc.) and immunological and health status. Differences between populations in the proposed experimental and control sites as well as the differences these sites might have from the larger regional population must be considered. Living conditions or behaviors associated with socioeconomic status, customs, and cultures can impact mosquito population sizes differentially, even when human population densities are equivalent. Ideal experimental and control sites feature populations with similar demographic features representing a cross-section of the wider population as a whole.

Presence of disease. The goal of controlling the vector species is to stop the spread, or potential spread, of a vector-borne pathogen. The site must be endemic for disease if the goal of the proposed field trial is to establish epidemiological end points or demonstrate control of disease. Presence or absence of the pathogen is not required for Ranging and Suppression Trials with purely entomological end points.

Additional considerations:

- Areas where the disease is at least potentially present could be better models for endemic areas.
- Disease-free sites may be preferable for some specific trial designs: *e.g.*, if the design requires a control site to remain untreated.
- Where epidemiological end points are to be considered, migration of humans into and out of the proposed trial area(s) is a critical consideration (See Isolation section, below). A less mobile and therefore insular community would potentially be preferable and allow the use of geographically smaller sites.
- Quality of public health monitoring/recording of disease, including availability of historical data.

Sizes of proposed sites. Large sites and large numbers of sites in a cluster-randomized design will improve the accuracy and validity of the study. However, these require more resources than smaller-scale efforts (see Resources section, below). Site size will be a trade-off between the demand for statistical robustness and costs. In practice, sites selected are as small as possible, while remaining large enough to deliver the objectives of the trials. Both the area (km^2) and the density of the human population will be parameters in this balance. Higher-density human populations typically support higher mosquito populations, and the vectorial capacity of a mosquito population is related more directly to numbers of mosquito/person, rather than numbers/area.

Pilot-Scale Intervention Trials and similar studies that include an epidemiological end point of disease reduction will generally have to involve larger human populations to measure an effect of the intervention. Treated and control areas will have to have sufficient disease cases to demonstrate statistically significant effects of intervention (Wolbers et al. 2012). Prevalence and incidence of disease within the population also will need to be considered. For example, a smaller population with a higher number of disease cases/month would likely be more suitable than a site with a larger population but with few or intermittent cases.

Duration of trial. Trials will have a temporal scale (duration) governed by factors, including the time needed to achieve trial outcomes. A Ranging Trial of fsRIDL should be of long enough duration to approach equilibrium in terms of the key end points of male-to-male ratios and egg paternity. This requires a temporal component that spans at least the mean life span of the released males, for the transgenic male population to approach equilibrium, plus the life span of wild adult females, so that females who mated before the equilibrium transgenic-to-wild ratio was reached have largely disappeared from the field population. This equates to a minimum trial durations of 4–6 weeks. For a Suppression Trial, models developed in support of a large laboratory cage trial (Wise de Valdez et al. 2011) indicated a minimum release period of 3–4 months to see an effect; therefore, 4–6 months would be a prudent minimum, especially since the end point is likely to be an indication of a sustained, rather than transient, effect.

There is a potential trade-off between spatial and temporal scales for trials in which epidemiological end points are anticipated. The total number of expected disease cases is a function of spatial (via the number of inhabitants included) and temporal scale (as well of other factors; *e.g.*, intensity of transmission). Seasonal effects are important in the evaluation of time commitments associated with trial designs. These effects influence the abundance of estivating embryos in the target area and affect both the optimum start time and the minimum/optimum duration of a trial.

Additional considerations that could impact the duration of the trial are:

- Political will (see Political environment section, below)
- Resources (see People and institutions section, below)
- ESC and managed expectations (see People and institutions section, below)

Isolation. Ideally, experimental and control sites should be isolated geographically from human dwellings not included in the trial. How much this matters depends on the strategy being tested, trial design, scale, and end points. Population replacement strategies that rely on gene flow also must consider field-site isolation as part of the risk assessment process. This isolation for population suppression strategies minimizes the impact of immigration of mosquitoes from adjacent sites. Although isolation is not necessarily required for the Ranging Trial, it is more important for a small-scale Suppression Trial. However, as the spatial scale becomes larger, the issue of isolation becomes somewhat less significant as migration effects are confined to border areas that become less significant relative to the total site area (*i.e,* the ''signal'' of suppression is stronger relative to the ''noise'' of immigration). Finally, the end points of the trial will dictate the level of isolation required. If initial vector population levels are relatively high and the desired end point is to show an impact on population densities, it is not likely that immigration due to lack of isolation would be high enough to prevent detecting this (although the reason for the presence of residual populations may be unresolved).

Ae. aegypti ranges typically <200 meters (Trpis et al. 1995, Harrington et al. 2005, Russell et al. 2005, Lacroix et al. 2012, Valerio et al. 2012). Site isolation by ≥ 400 meters should be more than adequate to be viewed as geographically isolated for *Aedes* populations for most purposes (Reiter 1995, Harrington et al. 2005). The isolating terrain must be of a type that the mosquitoes are unlikely to cross. Barriers include open terrain and water and uninhabited vegetated areas. Major highways have been shown to be barriers to movement (Hemme et al. 2010), but one alone likely would not be sufficient. If geographically isolated sites are not available, a potential alternative approach is the use of buffer zones around a selected test site, perhaps in one direction where isolation is considered inadequate. The buffer zone has to be an appropriate width to form a barrier to mosquito immigration from adjacent areas. Buffer zones receive treatment (using conventional or genetic-strategy control measures) and therefore isolate the test site, but are not included in the data analysis.

Control sites. Field trials should contain control sites, or have a comparable site nearby that can act as a control. We recognize that this will not always be feasible for large intervention programs. In these instances, the impact of intervention can be judged against historical patterns of vector population and disease prevalence.

Protected biotype and other significant resources. Genetic strategies are designed as species-targeted interventions with negligible off-target effects and are the ideal intervention to use in ecologically or culturally sensitive areas. However, it is prudent to locate initial trial sites away from protected biotypes, such as nature reserves and other protected biotypes or ecotypes, for example, threatened and endangered species or aboriginal communities.

Other vector-control activities. Genetic strategies are anticipated to be compatible or synergistic with most or all other control methods. fsRIDL should combine well with interventions that target immature stages or females. Interventions targeting adult males should be compatible as long as they do not target specifically fsRIDL males over wild males. fsRIDL also provides a resistance management

solution for other methods (*e.g.*, increasing the insecticide susceptibility in a population as an unlinked trait; Alphey et al. 2007, Alphey et al. 2009). However, conventional vector-control activities applied differentially among control and release sites will affect the usefulness of a comparative analysis between the sites.

Adverse natural conditions. The frequency and severity of known adverse natural conditions (hurricanes/typhoons, floods, mudslides, earthquakes, volcanoes) in the region of the proposed trial site(s) should be documented as part of the assessment of the suitability of the site. Seasonality of the potential weather events also impact trial design.

Adverse human activities. Human activities can have wide and diverse effects on field trials. These effects can result from normal behavior (*e.g.*, daily or seasonal migration patterns) or may be sporadic and unmanageable, such as crime (including willful sabotage) or civil unrest (Anonymous 1975, Lowe et al. 1980). These activities will affect trial design, resources requirements, and risk assessment (*e.g.*, human activities represent methods by which fsRIDL mosquitoes can be transported inadvertently out of the trial sites). Prior consideration of the potential for adverse human activities is not only useful in the evaluation of potential field sites but also informs trial design (*e.g.*, the design of monitoring activities).

Strategic planning. A phased series of trials of increasing scale can be anticipated, and data from earlier trials will inform later ones. The relevance/applicability of these early data will be greater if the later trials are in similar or the same locations. Even if resources for large-scale trials are not available initially, we encourage researchers to think strategically about whether proposed sites would be appropriate for subsequent experiments should additional support become available.

Regulatory requirements

A goal of the site-selection process is to identify locales where regulatory pathways exist and have the capacity for oversight of activities associated with research of genetically engineered mosquitoes (*i.e.*, review and approval of trial designs, issuing permits for importation and release, monitoring ongoing trial activities, and evaluating and responding to reports of trial conclusions and any adverse events). This process can be challenging but is a critical criterion in the site-selection process (see Box 1, above, and Table S2). Without legislation and a regulatory structure in place, the research trials run the risk of being prevented or halted prematurely. More importantly, public confidence is based in part on the credibility of the regulatory process. Perceived failings in this area risk negative impact on community perception and that of decision-makers in other potential sites, diminishing research opportunities for the development of strategies with potentially substantial public health impacts.

Genetically engineered mosquitoes

Legislation and permitting. Importation, local research and release of genetically engineered mosquitoes and other project activities require permits from regulatory authorities. Permitting may be a function of the central government (federal) or delegated to a regional authority (for example, state, county, and municipal agencies). In addition, the capacity and authority of the collaborating organizations to initiate, carry out, and fulfill the regulatory and compliance requirements must be assessed.

Regulatory process. Refinement and capacity building of regulatory pathways may be required to address specific issues associated with field trials of transgenic mosquitoes. This likely will be the case, even under circumstances where a regulatory structure is in place to oversee research and use of other (noninsect) genetically engineered organisms. A long-term perspective is important if the research program participates in pathway development. For example, while it is essential to meet all of the requirements appropriate for a thorough and complete evaluation process, it also is important to not set a precedent for unnecessary or overly burdensome oversight for future trials.

Many aspects of the regulatory process will be country specific, and the necessary permits and the processes for obtaining them differ significantly among them. Less variability is expected among potential trial sites within the same country, although responsible agencies likely will require specific conditions to be met for each site as a requirement for granting regulatory approval. Compliance with such conditions is an important part of conducting research with regulated materials. Evidence should be sought of the political will to further define and refine these pathways when needed.

Other regulated research. Trials of genetic strategies will involve research activities subject to other national or local approvals. Research involving infectious agents, hazardous materials, recombinant DNA molecules, or gene transfer must obtain appropriate institutional approvals. Vertebrate animals used for blood feeding must be provided with humane care that meets standards set by all institutions involved. Human subject research must comply with relevant aspects of the Declaration of Helsinki, Belmont Report, International Conference on Harmonization (ICH) guidelines, or the Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines for Biomedical Research involving Human Subjects. All studies involving human subjects must meet Good Clinical Practice standards (www.fda.gov/oc/gcp/guidance.html) or other similar specific human subject protections required to fulfill the national or international standards (*e.g.*, Organisation for Economic Co-operation and Development, www.oecd.org/sti/sci-tech/49344626.pdf). Assessments include whether oversight from institutional boards is needed from the funded institution and/or whether these boards may act as a surrogate if collaborating institutions are not equipped to oversee these types of research activities.

Community engagement (ESC considerations)

Collaborating with scientists with local ties is essential but not necessarily sufficient to introduce novel strategies and perform field trials in ways that are respectful and adhere to local ESC standards. The site-selection process should evaluate the capacity to put in place meaningful and productive community engagement activities (Table S3; Lavery et al. 2008, Lavery et al. 2010). Performing trials in ways that

meet these standards does more than determine the scientific and technical proficiencies of a novel strategy, it helps increase community acceptance and trust of the research findings and conclusions. Positive public perception and acceptance is as significant for trial success as meeting technical performance specifications.

Communication

Residential communities. The community will be defined and redefined in many ways in the site-selection process and during trial activities (Lavery et al. 2008). We adopted a protean definition of relevant communities as those that have a direct stake in the research (Brunger and Weijer 2007, Lavery et al. 2010). These components can be categorized in terms of individuals, groups, organizations, and agencies that have legitimate interests in the research. Examples include the teams of multinational and multidisciplinary scientists, administrators, agencies and oversight committees, officials and leaders of government and nongovernment agencies, and the local trial-site residents. The local residents are the most vulnerable of these groups, and specific efforts must be made to earn and foster authentic respect and trust.

Collaborators or collaborating institutions (*e.g.,* public health institutions) with proven track records and who have established trust among the residents of the communities are essential. These collaborators will not only help gauge local understanding of the role of vector control in preventing the spread of dengue, but will facilitate initiation of community engagement activities. These need to be started as early as possible to inform the local residents of the purpose and goals of proposed trials. It is important to identify pre-existing lines of communication and the ability to generate new networks where they do not exist. Open communication empowers the local community allowing them to interact in a meaningful way with the research team, access relevant information and express their opinions.

Communication plan. Field-trial projects should include both reactive and proactive communication plans. The reactive plan allows for timely and unified research team responses to questions from the community and others that are likely to arise. The proactive plan reaches out to target audiences informing them of the specifics of the trials. The proactive plan should communicate efficiently and effectively the goals of the scientists and the trial and keep the community informed of progress. A plan should take into consideration all community stakeholders, including the developers, scientific oversight committees, regulators, collaborators, and other program stakeholders. Assessing current communication plans and target audiences can identify the current level of capacity and highlight any gaps that exist.

Working environment. The history and previous experiences with genetically engineered organisms and/or vector control, as well as the current political landscape, are important considerations when trying to determine whether the trials will likely receive ongoing support from relevant communities, authorities, and decision makers.

Political environment. Enthusiasm and support for field trials can be characterized geographically, temporally, and politically. Understanding the political structures and how these structures interact over the course of the trials can help to determine whether these factors could impact the successful completion of the trial. Support that is too local or defined too narrowly can expose potential risks that may arise as inevitable changes in the political landscape occur. Understanding how and when these changes arise (*e.g.*, the date of the next regularly scheduled election) can help to determine the likelihood that they would impact future studies.

ESC considerations. Many aspects of ESC issues are hard to quantify or define; however, framing ESC issues is critical to understanding whether the proposed activities have potential value to the trial stakeholders (Lavery et al. 2008, Lavery et al. 2010). Understanding attitudes is crucial at all stages of the trials including the potential future uptake of the technology.

Resources

The breadth and scope of field trials will depend largely on the resources that are available to support its activities. An assessment of a potential field site should include compiling an inventory of available resources and an evaluation of the quantity, quality, and extent to which these resources can be committed (Table S4). Resources needed for field trials include time, money, expertise, facilities, and personnel (trust also is a valuable resource). Resource requirements for a given experimental plan are likely to vary considerably from one country or location to another.

People and institutions.

Successful open-release trials are dependent absolutely on the availability of expertise and the level of commitment to the project from the collaborators, collaborating institutions, government officials, and available personnel. Understanding the motivations of stakeholders can be difficult to ascertain but can help gauge the level of commitment by collaborators working directly on the project and the level of support and backing they can anticipate throughout the trials. Understanding these motivations also can aid in managing expectations as part of Community Engagement.

Some field-trial activities can be supported from off-site locations, whereas others require facilities and expertise on or adjacent the proposed trial sites. Local research institutions that have experience with entomology/mosquito vector research and who have a history of participation in international collaborations are a valuable asset. Local expertise must be identified in multiple disciplines but also must exist within oversight and advisory committees.

Identifying skill-set gaps and training needs is as important as understanding the expectation of resource allocation among collaborators (at the field site and beyond). There may be strong preferences to support activities at or near the site regardless of whether optimal resources exist elsewhere. Understanding these expectations as early as possible can help to avoid miscommunication and prevent later conflicts.

Time is an important and highly valuable resource. This component includes both the percentage of time each key person can commit on an averaged daily basis and how long they can commit this level of effort to the project. Personnel appointments likely are sensitive to political or institutional backing, contractual obligations or unstable or limited funding

Financial/employment. Field trials require significant funding. Costs for working at specific sites will be associated with the local economy (or cost of living), and a reconciliation of the projected costs can help to identify the scope of the trial or whether there is sufficient funding to support a trial. Employee compensation (salaries and benefits) is likely to make up the bulk of the costs associated with a research trial. Other costs associated with finding and hiring skilled workers should be explored.

Additional costs can be expected to include import/export fees, material shipping costs, travel-related expenses, regulatory oversight fees, insurance, and value-added taxes. Some of these costs could be paid by the collaborating institutions or offset by collaborating with local mosquito-control authorities and agencies.

Logistics. An assessment of the logistics associated with field trials includes operational activities, such as the provision, location, storage, protection, and deployment of project resources. Analyses of these factors will identify resource gaps and requirements for field trial activities. Logistics will be impacted by legal requirements, local infrastructure, distances (between field-sites and between field-sites and facilities), access to reliable utilities, local languages, and worker-safety concerns.

Collaborator resources. Local collaborators may be able to contribute financial or in-kind resources to the project from internal resources or third-party funding. Examples include access to facilities (laboratories, insectaries, offices), assignment of personnel to the project at no or reduced cost to the project (*e.g.*, principal investigators, pre- and postdoctoral researchers, technicians, statistician, secretarial, administrative, and transport support staff), use of vehicles, laboratory or other equipment, translation services, and support services such as information technology, payroll, legal, etc.

Summary

Establishing site-selection criteria for field trials of genetically engineered mosquitoes supports efforts to choose appropriate trial sites based on four categories (Scientific, Regulatory, Community Engagement, and Resources). While the criteria here are provided to assist laboratory scientists in their phased testing of genetic control strategies, they also are a starting point for open dialogue and communications prior to establishing strong collaborative ties and initiating community engagement activities. These criteria are expected to stimulate further discussion and critical analysis and to support the development and refinement of best practices for establishing field trials. Although these criteria can serve as guidance for establishing a site-selection process, we recognize that the process will be influenced by factors unique to the strategy being tested, the conditions under which the strategy is to be tested, and the purpose and goals of the trials.

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References

Alphey N, Coleman PG, Donnelly CA, Alphey L. Managing insecticide resistance by mass release of engineered insects. J Econ Entomol 2007; 100:1642–1649.

Alphey L, Nimmo D, O'Connell S, Alphey N. Insect population suppression using engineered insects. In: Aksoy S, ed. *Transgenesis and the Management of Vector-Borne Disease*. Austin, TX: Landes Bioscience, 2008:93–103.

Alphey N, Bonsall M, Alphey L. Combining pest control and resistance management: Synergy of engineered insects with Bt crops. J Econ Entomol 2009; 102:717–732.

Anonymous. Oh, New Delhi; Oh, Geneva. Nature 1975; 256: 355–357.

Benedict M, D'Abbs P, Dobson S, Gottlieb M, et al. Guidance for contained field trials of vector mosquitoes engineered to contain a gene drive system: Recommendations of a scientific working group. Vector Borne Zoonotic Dis 2008; 8: 127–166.

Black WC 4th, Alphey L, James AA. Why RIDL is not SIT. Trends Parasitol 2011: 8:362–370.

Brunger F, Weijer C. Politics, risk, and community in the ICBG-Chiapas case. In: Lavery JV, Wahl ER, Grady C, Emanuel EJ, eds. *Ethical Issues in International Biomedical Research: A Casebook.* New York: Oxford University Press, 2007:35–42.

Chanda E, Hemingway J, Kleinschmidt I, Rehman AM, et al. Insecticide resistance and the future of malaria control in Zambia. PLoS One 2011; 6:e24336.

Cohen J. Public health. Gates Foundation picks winners in Grand Challenges in Global Health. Science 2005; 5731:33–35.

Collins FH, James AA. Genetic modification of mosquitoes. Sci Med 1996; 3:52–61.

Curtis CF. Possible use of translocations to fix desirable genes in insect pest populations. Nature 1968; 27:368–369.

Facchinelli L, Valerio L, Bond JG, Wise de Valdez MR, et al. Development of a semi-field system for contained field trials with *Aedes aegypti* in southern Mexico. Am J Trop Med Hyg 2011; 85:248–256.

Facchinelli L, Valerio L, Ramsey JM, Gould F, et al. Field cage studies and progressive evaluation of genetically-engineered mosquitoes. PLoS Negl Trop Dis 2013:e2001.

Focks DA, Barrera R. Working Paper 6.1. Dengue Transmission Dynamics: Assessment and Implications for Control. In: World Health Organization, Scientific Working Group Report on Dengue [online], Geneva, Switzerland: World Health Organization, 2007:92–109. Available at www.who.int/tdr/publications/documents/swg_dengue_2.pdf

Franz AW, Sanchez-Vargas I, Adelman ZN, Blair CD, et al. Engineering RNA interference-based resistance to dengue virus type 2 in genetically-modified *Aedes aegypti*. Proc Natl Acad Sci USA 2006; 14:4198–4203.

Fu G, Lees RS, Nimmo D, Aw D, et al. Female-specific flightless phenotype for mosquito control. Proc Natl Acad Sci USA 2010; 9:4550–4554.

Harrington LC, Scott TW, Lerdthusnee K, Coleman RC, et al. Dispersal of the dengue vector *Aedes aegypti* within and between rural communities. Am J Trop Med Hyg 2005; 72:209–220.

Harris AF, Nimmo D, McKemey AR, Kelly N, et al. Field performance of engineered male mosquitoes. Nat Biotech 2011; 29:1034–1037.

Harris AF, McKemey AR, Nimmo D, Curtis Z, et al. Successful suppression of a field mosquito population by sustained release of engineered male mosquitoes. Nat Biotech 2012; 30:828–830.

Hemingway J, Beaty BJ, Rowland M, Scott TW, et al. The Innovative Vector Control Consortium: Improved control of mosquito-borne diseases. Trends Parasitol 2006; 22:308–312.

Hemme RR, Thomas CL, Chadee DD, Severson DW. Influence of urban landscapes on population dynamics in a short-distance migrant mosquito: Evidence for the dengue vector *Aedes aegypti*. PLoS Negl Trop Dis 2010; 4:e634.

James AA. What's that buzz? Mosquitoes and fruit flies commingle. Parasitol Today 2000; 16:503–504.

James AA, Benedict MQ, Christophides GK, Jacobs-Lorena M, et al. Evaluation of drive mechanisms (including transgenes and drivers) in different environmental conditions and genetic backgrounds. In: Knols BGJ, Louis C, eds. *Bridging Laboratory and Field Research for Genetic Control of Disease Vectors.* Dordrecht, The Netherlands: Springer, 2006:149–155.

James S, Simmons CP, James AA. Ecology. Mosquito trials. Science 2011; 6057:771–772.

Kilpatrick AM, Randolf SE. Drivers, dynamics, and control of emerging vector-borne zoonotic diseases. Lancet 2012; 9857: 1946–1955.

Knols BGJ, Bossin H. Identification and characterization of field sites for genetic control of disease vectors. In: Knols BGJ, Louis C, eds. *Bridging Laboratory and Field Research for Genetic Control of Disease Vectors.* Dordrecht, The Netherlands: Springer, 2006:203–209.

Labbé GM, Scaife S, Morgan SA, Curtis ZH, et al. Female-specific flightless (fsRIDL) phenotype for control of *Aedes albopictus*. PLoS Negl Trop Dis 2012 7:e1724.

Lacroix R, McKemey AR, Raduan N, Kwee Wee L, et al. Open field release of genetically engineered sterile male *Aedes aegypti* in Malaysia. PLoS One 2012; 8:e42771.

Lavery JV, Harrington LC, Scott TW. Ethical, social, and cultural considerations for site selection for research with genetically-modified mosquitoes. Am J Trop Med Hyg 2008; 79:312–318.

Lavery JV, Tinadana PO, Scott TW, Harrington LC, et al. Towards a framework for community engagement in global health research. Trends in Parasitol 2010; 26:279–283.

Lowe RE, Bailey DL, Dame DA, Savage KE, et al. Efficiency of techniques for the mass release of sterile male *Anopheles albimanus* Wiedemann in El Salvador. Am J Trop Med Hyg 1980; 29:695–703.

Marinotti O, Jasinskiene N, Fazekas F, Scaife S, et al. Development of a population suppression strain of the human malaria vector mosquito, *Anopheles stephensi*. Malar J 2013; 12:142.

Mathur G, Sanchez-Vargas I, Alvarez D, Olson KE, et al. Transgene-mediated suppression of dengue viruses in the salivary glands of the yellow fever mosquito, *Aedes aegypti*. Insect Mol Biol 2010; 19:753–763.

Milani R. The genetics of *Musca domestica* and of other muscoid flies. In: Wright JW, Pal R, eds. Genetics of Insect Vectors of Disease. Amsterdam, London, New York: Elsevier Publishing Company, 1967:343–350.

Nawtaisong P, Keith J, Fraser T, Balaraman V, et al. Effective suppression of Dengue fever virus in mosquito cell cultures using retroviral transduction of hammerhead ribozymes targeting the viral genome. Virol J 2009; 4:73.

Phuc HK, Andreasen MH, Burton RS, Vass C, et al. Late-acting dominant lethal genetic systems and mosquito control. BMC Biol 2007; 5:11.

Reiter P, Amador MA, Anderson RA, Clark GG. Short report: Dispersal of *Aedes aegypti* in an urban area after blood feeding as demonstrated by rubidium-marked eggs. Am J Trop Med Hyg 1995; 52:177–179.

Russell RC, Webb CE, Williams CR, Ritchie SA. Mark–release–recapture study to measure dispersal of the mosquito *Aedes aegypti* in Cairns, Queensland, Australia. Med Vet Entomol 2005; 19:451–457.

Tee AS, Daud AR, Alias M, Lee HL, et al. Guidelines on the use of ovitrap for *Aedes* surveillance. Vector Control Unit, Vector-Borne Disease Section, Ministry of Health, Malaysia, 1997:6 pp.

Thomas DD, Donnelly CA, Wood RJ, Alphey LS. Insect population control using a dominant, repressible, lethal genetic system. Science 2000; 287:2474–2476.

Trpis M, Häusermann W, Craig G Jr. Estimates of population size, dispersal, and longevity of domestic *Aedes aegypti aegypti* (Diptera: Culicidae) by mark-release-recapture in the village of Shauri Moyo in eastern Kenya. J Med Entomol 1995; 32:27–33.

Valerio L, Facchinelli L, Ramsey JM, Scott TW. Dispersal of male *Aedes aegypti* in a coastal village in southern Mexico. Am J Trop Med Hyg 2012; 86:665–676.

Wise de Valdez MR, Nimmo D, Betz J, Gong H.F, et al. Genetic elimination of dengue vector mosquitoes. Proc Natl Acad Sci 2011; 22:4772–4775.

World Health Organization/Tropical Disease Reasearch. Progress and prospects for the use of genetically-modified mosquitoes to inhibit disease transmission. Report on planning meeting 1: Technical consultation on current status and planning for future development of genetically-modified mosquitoes for malaria and dengue control, 2010. WHO/TDR publications. Available at www.who.int/tdr/publications/training-guideline-publications/gmm-report/en/index.html

Wolbers M, Kleinschmidt I, Simmons CP, Donnelly CA. Considerations in the design of clinical trials to test novel entomological approaches to dengue control. PLoS Negl Trop Dis 2012; 6:e1937.

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Supplementary Data

SUPPLEMENTARY TABLE S1. SCIENTIFIC

Entomological data

Presence of target species (*Aedes aegypti*)

- Is the target species present at selected sites (proposed release and control sites)?
- What data are available on current and historical numbers of the target species at each location (many types of data are relevant and 'ideal' information may not be available)?
- How do those population sizes fluctuate through the different seasons of a year?
- What is the history of the target species and how have populations changed over time (again, many types of data are relevant and 'ideal' information may not be available)?

Presence of other mosquito species

- Are other mosquito species present?
- Which species appear and in what estimated or relative numbers in monitoring systems to be used (from any trapping system, but particularly from ovitraps, BG-Sentinels and backpack aspirators).

Human inhabitants

- What are the approximate numbers and demographics of the human population(s) of proposed release and control sites?

Presence of disease

- Describe the nature of dengue transmission at the proposed release trial site(s), including possible transmission by species other than *Ae. aegypti*.
- What is the history of dengue at the proposed locations? (Dengue incidence by year, season, location; is there laboratory confirmation of dengue fever and dengue hemorrhagic fever; age-specific incidence; circulating serotypes)
- What is the status of chikungunya and yellow fever viruses in the proposed sites?
- Describe the availability of facilities for monitoring dengue incidence (e.g. medical clinics, clinical research facilities) at the proposed sites, and how these are funded.
- What are the most frequent illnesses and top medical issues for people in the target community? How often are these confused with dengue fever?

Size(s) of proposed sites

- Describe the scale of site(s) in terms of geographic area (hectares or km^2). Mosquito and human populations are described in text (Entomological Data and Human inhabitants sections, respectively).

Duration of trial

- What is the capacity for commitment of the DEC partners to the duration of the trial?

Isolation

- Are the proposed sites geographically isolated by ≥ 400 meters?
- What is the nature of the isolating terrain or other barrier(s) separating the mosquito population at the site from other known or suspected mosquito populations?
- If the sites have ≤ 400 meters isolation, what mitigations are available, *e.g.*, barrier treatment in buffer zone?

Control sites

- Can a control site be identified for each potential trial site?
- To what extent are they similar and different to the proposed release sites in aspects relevant to the trial (or to what extent are there a set of comparable sites to be used either for release or as controls)?

Protected biotype and other significant resources

- Survey and describe the protected biotypes/ecotypes and other sensitive areas that are within or close to the proposed sites.

Other vector control activities

- What vector control operations are conducted in the area?
- Who is responsible for these activities?
- How are these activities funded (through which agencies/bodies of government)?
- Who are the contact people for each relevant agency?
- What is known of their program or intentions for treating (or otherwise) the proposed trial sites? Please refer to the questions in the Regulatory section about statutory or compulsory treatment.

Adverse natural conditions

- Describe the proposed site environment in terms of risk of adverse natural conditions. As appropriate, this should include locations containing laboratory facilities, access routes *etc*., as well as the proposed release sites themselves.

Adverse human activities

- What is the distance to roads, railroads, airports and other possible means by which released mosquitoes can be transmitted by humans out of the trial sites?
- Is there a real threat of civil unrest, nationally or in proposed trial area(s)?

Next steps

- If the planned trial is successful, what are the prospects for moving to a larger scale at the same or nearby site?

SUPPLEMENTARY TABLE S2. REGULATORY REQUIREMENTS

Genetically engineered mosquitoes

Legislation and permitting

- Are regulations/legislation in place governing research and other activities with recombinant DNA, etc.?
  - If so, what are the key relevant regulations/legislation?
- What is the status of the Cartagena Protocol on Biosafety (http://bch.cbd.int/protocol/) in the country and how is it being implemented?
  - If relevant, identify the in-country CPB contact point.
- Were any relevant legislation or policies in place prior to Cartagena obligations and what is their current status?
- Are there any specific laws/norms/guidance, etc., regarding vector control where project activities (*e.g.*, baseline monitoring/surveying) might interact with these regulations?
  - Any conventional control plans (*e.g.*, IVM, barrier treatment, risk mitigation plans) will need to take account of any such regulations.
- Are there other known regulatory/legal issues that may affect project operations? Examples may include:
  - Restrictive customs regulations or delays,
  - Immigration (visas, work permits),
  - Restrictions on exporting samples (for example, biodiversity/bioprospecting law).

Regulatory processes

- Has the country had previous experience importing GE mosquitoes for laboratory research?
  - If not, what about other GE insects (*e.g., Drosophila* for research purposes), plants or other animals?
- What are the relevant national, state, municipal and local agencies and their specific roles?
- Is there a clear process for application and approval for the proposed research?
  - If so, provide an outline of the structure and process.
  - What are the estimated time-lines for completing each of the regulatory steps?
  - What is the basis for this estimate?
- Is a risk assessment/risk management plan required? If so, who develops it, the applicant or the authority?
- What are the opportunities/mechanisms/requirements for public engagement in the regulatory process?
  - Some regulatory processes are fully confidential, some open to the public, some are a mix (for example, applications become publicly available but information identified as confidential by the applicant is redacted).
- What are the inspection and audit regimens for compliance with granted permits?
  - These may be described in the legislation, but in some instances may not be known in advance of the permit being granted, for example, they may be attached as conditions of permits.
- What are the internal approval procedures of the proposed in-country collaborating institution?
  - Are there precedents for prior use of these procedures?
  - What committees/structures are involved?
  - Is this a public or confidential process?
  - Are there interactions with governmental approval/permitting processes? With ethical, social, and cultural?
  - Is there sufficient capacity for regulatory compliance and reporting?

Other regulated research

- Describe the Institutional Review Boards (IRBs)/Institutional Ethics Committee (IECs) responsible for oversight of Human Subjects research at the institutions to be involved in the trials.
- Describe the Institutional Biosafety Committees (IBC) or equivalent review bodies responsible for oversight of research involving biohazardous agents.
- Describe the Institutional Animal Care and Use Committee (IACUC) or equivalent institutional review bodies responsible for oversight of research involving vertebrate animals.
- Does the country have national guidelines for research with recombinant DNA?
- Does the in-country collaborating institution have an established research review procedure for research with recombinant DNA?
  - If so, what are the procedures, structures, informational requirements and projected timescale(s)?

SUPPLEMENTARY TABLE S3. COMMUNITY ENGAGEMENT (ETHICAL, CULTURAL, AND SOCIAL)

Communication

Residents

- What are the mechanisms and conditions to facilitate interface with the trial-site residents and basis for ethical, cultural, and social (ESC) collaborations?
  - Describe any previous interactions of the collaborators with the community at the proposed trial site.
  - Is there an ongoing relationship of trust with the public health agencies and institutional scientists?
  - Describe proposed methods for informing and involving the community in preparation for trials. How would community opinions influence planning for the trials?
  - Is there any precedent or other evidence for the likely effectiveness of these methods?
- How is community engagement for vector control normally conducted in the country?
  - What is the level of understanding by communities in the proposed trial sites regarding dengue and the role of mosquitoes in transmitting dengue?
  - What process is used by public health authorities for informing the community about vector control activities? Is a mechanism for community feedback in place?

Communication Plan

- A Communications Plan will need to be put into place for both proactive and reactive communications.
  - Have stakeholders/influencers in the country and local area been identified?
  - Has a Communications Plan been developed or is one in the process of being developed?
    - At early stages this may be only in draft, outline or partial form.
- What is the local working language?
  - Do the in-country collaborators speak this language or dialect? Visiting scientists?
  - Is translation available as necessary?

Working environment

Political environment

- **What is the political system in the country/location? Relevant issues may include:**
  - Levels of government (*e.g.,* federal/state/municipal)
    - Is there conflict between levels of government likely to impose difficulties?
  - Is the political system relatively stable?
    - Are there imminent elections that may disrupt civil service (*e.g.,* collaborators or regulators), or lead to significant shifts in policy (*e.g.,* on GE organisms)?
  - If the country/site has an electoral process, when is the next election that could have a relevant impact on the proposed studies?
  - Who are the relevant authorities in the proposed field site? What are their mandates (may include health/environmental authorities, general government, etc.)?
  - Describe the cultural leadership structure of the community (are there village chiefs, groups of elders, respected religious figures and other nongovernmental figures of respect and authority?).
- Is there a political will to embrace new solutions for dengue control?
  - Evidence?
- What is the political/community history with introductions of GE organisms in the country?
  - What is the level of understanding by communities in the proposed trial-site region regarding genetic engineering?
  - Have there been previous interactions with non-governmental agency or advocacy group?
    - Components may include an overall plan/posture, key messages, frequently asked questions (FAQ) and other question and answer documents, and include local opinion leaders and third-party spokespersons.

ESC considerations

- If relevant, who has property rights at the proposed field-site location(s)?
  - Is there any risk that the research would displace individuals or communities at the preferred site(s)?
  - Is the proposed site located near to valued community resources or sites where vulnerable populations might be impacted by the trials (*e.g*., hospitals, recreational areas, schools)?

SUPPLEMENTARY TABLE S4. RESOURCES

People and institutions

- What are the reasons for interest in an open-release trial?
- What are the expectations of the collaborators?
- Provide the identity and nature of the proposed in-country collaborator and collaborating institution(s)?
  - Institution type (for example, government/university/private sector), size, identity and mission.
  - Previous history of international collaboration? With other project members?
  - Identify key individuals, for example the Principal Investigator, institute director, regulatory officer (if known)
    - Provide the background and relevant experience of key personnel.
    - What are their other duties, and how does this affect the time they have available for project?
  - As applicable, identify skill sets, gaps and training needs
  - Are there any known or foreseeable circumstances under which the institution or individuals might not be able to continue to collaborate for the full duration of project (*e.g.*, short-term contracts, institution reorganizing, unstable mission/activities/funding)

Financial/employment

- What policies govern recruitment, hiring, human resources management issues (practices and accountability) in the collaborating institution?
- What are the relative costs of operating in the country/region?
  - Cost-of-living (*e.g.*, relative to the United States or United Kingdom).
  - Indicate typical salaries for different grades (for example, graduate students, technicians, postdoctoral fellows, principle investigator other project personnel).
  - Are travel costs (flight, subsistence) and times unusually high or low?
- Any known additional costs
  - Compulsory benefits, bonuses or insurance, other employment law?
  - Regulatory costs ('user pays') and time?
  - Value Added Taxes (VAT)?

Logistics

- How far in distance is it from the collaborator's facility to the proposed field site(s)?
  - Journey time, cost, transport resources
  - Is there safe, reliable access to proposed facilities (*e.g.*, laboratories, field site and travel to/between them)? (see article sections regarding adverse natural and adverse human conditions).

Collaborator resources

- What resources does the in-country collaborator propose to provide to the project?
  - Of these, what will be funded by the collaborator, what from third-party sources and what will need to be provided from the project? Project contribution likely will be a mix of in cash and in kind, *e.g.*, provision of personnel, etc.
  - What capacity will be made available to the project to handle regulatory issues (permitting, inspections and compliance); for example, will the collaborating institutions provide people and expertise to oversee applications through the committees?
  - What is the previous experience and evidence of this?
  - What is the capacity to manage epidemiological surveillance and treatment of dengue and dengue hemorrhagic fever?
- What are the co-sponsorship opportunities?
  - Private foundations
  - Government grants
  - Resource-sharing with current vector control efforts
  - Who is eligible for applying and administering these opportunities?