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"The Infravec2 Infrastructure Project: Providing Vector Researchers with No-cost Resources, Services and Facility Access"

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Abstract

The Infravec2 infrastructure project provides insect vector resources and facility access to researchers worldwide at no cost. The project is funded by the European Commission Horizon 2020 Research Infrastructure Program (INFRAIA). The 24 Infravec2 partners operate major European biosecure insectaries for experimental infection and containment of insect vectors and other key insect vector technology platforms including front-line field sites in Africa, the Pacific, and the Americas. Researchers can shop online and request resources from an extensive product catalog (www.infravec2.eu). Infravec2 is also developing innovative new research tools, providing training courses, and is networking the community with activities such as common experimental standards and protocols to obtain reproducible vector infection results across different facilities. Infravec2 is a source of EU research support for vector researchers. The Infravec2 goal is to accelerate European innovation in basic and translational insect vector biology, and to consolidate a high-quality insect vector infrastructure with long-term perspectives for improving global public health. Key Words: Vector Biology, Tsetse fly, Infection Experiments, Training, Public Health.

"Increasing insecticide resistance in malaria vectors of the An. gambiae complex in Tanzania"

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Abstract

Understanding the dynamics of insecticide resistance in African malaria vectors is crucial for successful implementation of resistance management strategies. The present study is aimed at investigating the temporal dynamics of insecticide resistance in An. gambiae s.l. populations from mainland Tanzania.

Methods: Mosquito larvae were collected across 30 sites of Tanzania mainland from 2010 to 2019. Phenotypic resistance was determined using standard WHO susceptibility tests. Molecular identification of An. gambiae species complex and kdr molecular genotyping was perform by PCR based techniques. Detection of metabolic resistance was realized using PBO-synergists tests and CDC microplate bioassay. Results: Susceptibility status of Anopheles gambiae s.l. to permethrin, deltamethrin, lambdacyhalothrin, bendiocarb and DDT showed a declining trend over the period of 10 years. The resistance to all pyrethroids (permethrin, deltamethrin and lambdacyhalothrin) has been significantly increasing and spreading with time. Considering each site and time as a single data point and estimating the trend over time, there has been statistically significant negative correlation between post insecticide exposure mosquito mortality and year ($p < 0.05$). Anopheles gambiae s.l. showed similar downward trend in susceptibility to bendiocarb and DDT, although this was not statistically significant ($p > 0.5$). Anopheles gambiae s.l. exhibited high level of susceptibility to the organophosphate insecticides (fenitrothion and pirimiphos-methyl) at all sites tested. There was considerable kdr-1014S allelic frequencies variation among sites and years in Anopheles gambiae s.l. Generally, the kdr-1014S has been spreading and their frequencies increasing over time. Mixed function oxidase was found to be associated with pyrethroid resistance. Conclusion: This study revealed a rapid increase in pyrethroid resistance in An. gambiae s.l. population from all sites sampled; increases in kdr-1014F allelic frequencies were also observed over time. Findings call for strengthening malaria vector surveillance as well as the urgent need for developing insecticide resistance mitigation strategies.

"A comprehensive testing cascade to identify resistance breaking repurposed insecticides for next generation vector control tools: screening a panel of chemistries against *Anopheles gambiae*"

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Abstract

With insecticide resistance in malaria vectors spreading in geographical range and intensity, there is a need for compounds with novel modes of action to maintain the successes achieved by insecticide treated nets and indoor residual sprays. Repurposing existing insecticides, predominantly those developed for use in agriculture, is less risky than screening and chemical synthesis programmes for novel compounds and may be a rapid way to identify active ingredients of value to public health. Insecticides and acaricides from all IRAC classes including those with unclassified modes of action were considered for inclusion in a laboratory bioassay testing cascade against adult female *Anopheles gambiae*. A longlist of representative candidate compounds was selected, excluding those with safety concerns, unsuitable physiochemical properties, and those likely to be challenging to register for public health use. An initial screen using topical application eliminated compounds with insufficient intrinsic activity, and a tarsal assay identified those with activity at an appropriate concentration. Inclusion of an adjuvant enhanced the efficacy of several compounds, saving chemistries with great potential, given suitable formulation, from exclusion. The remaining shortlist

of compounds of interest as vector control agents were ranked by relative potency using dose response assays and discriminating dose calculations.

"A game changer in malaria vector control training: The serious game as an innovative training tool for effective knowledge transfer"

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Abstract

In the current climate of malaria elimination with widespread insecticide resistance compromising the effectiveness of vector control efforts, there is a new impetus for country programmes, international organisations and stakeholders working in vector control and surveillance to focus on enhanced learning and capacity building approaches in-country. To address this, LSTM has developed a novel approach to improve Insecticide Resistance Management (IRM) competencies among vector control staff, implementing partners and stakeholders.

We designed an IRM training course underpinned by innovative game-based learning. A 2.5-day course was structured around two serious digital games: *Resistance101*, an arcade-style game incorporating the fundamentals of insecticide resistance, and *ResistanceSim*, a simulation game based in a fictitious African country centred on implementing IRM strategies. Game play was supplemented by an interactive course structure that included mini-lectures, discussions and group exercises.

Under the umbrella of the DMC-MalVec Consortium, game-based insecticide resistance management training courses for disease control programme staff and academics were delivered and evaluated in Ethiopia and Zambia in 2017 and subsequently in Cameroon and Cote d'Ivoire in 2019. Questionnaires, knowledge tests, semi-structured interviews and focus group discussions were used to explore how game-based training contributed to the transfer and retention of knowledge and skills to the work environment**.**

Trends in increased knowledge and self-efficacy were observed. Participants highlighted the novelty and appeal of game-based training compared to historically didactic teaching methods. The design and structure of both games were reported as key learning features by participants. The architecture of *ResistanceSim* supports improved understanding of IRM activities and contextualizes participant roles, stimulating improved problem-solving and enabling better working practices.

Our findings provide evidence of the effectiveness of dynamic, game-based training sessions in the field of vector control whilst providing insights for the design of future courses including novel technologies and games as educational tools.

"Upsurge of malaria transmission after Indoor Residual Spraying withdrawal in Atacora region in Benin, West Africa."

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Abstract

In Benin, malaria vector control mostly relies on LLINs, and IRS operations. From 2011 to 2016, IRS program has been implemented in Atacora region. However, in 2017 the program was withdrawn from two other regions in the northern part of the country, with hopes that gains would relatively be sustained because of the seasonality of malaria transmission. What would be the vulnerability of populations to malaria after the withdrawal of IRS? Monthly mosquito collections were performed through human landing captures (HLCs) for twenty four months (from January to December 2016 during the last IRS campaign, and from January to December 2018 two years after the withdrawal of IRS). Vectors mosquitoes biting density was sampled by human landing collection (HLC) and were tested for presence of Plasmodium falciparum sporozoites. Moreover, the carcass of these mosquitoes (abdomens, wing and legs) were subjected to molecular species identification using polymerase chain reaction (PCR) assays. Results: We notice a drastic increase (~3 times higher) of vectors abundance after the withdrawal of IRS. Mosquito biting rates in the three survey districts increased significantly after IRS was withdrawn. In 2018, after IRS cessation it's recorded a significant increase of EIR where each inhabitant would receive an average of 94.9 infected bites/year to 129.21 infected bites/year against an average of 17.15 infected bites/year to 24.82 infected bites/year in 2016.

It is obvious that the withdrawal of IRS confers a vulnerability of the population with regard to the malaria transmission. Robust monitoring is needed to better understand when and where IRS should be most adequate, or can be safely withdrawn. In case of withdrawal, adapted accompanying measures should be proposed according to the contexts not only to maintain the gains capitalized with IRS, but also to avoid any rebound of transmission.

Non-Inferiority testing of Insecticide Treated Nets (ITNs): comparison of results from experimental huts and Ifakara Ambient Chamber tests

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Abstract

Candidate Insecticide Treated Nets (ITNs) with a claim of improved control of vector borne disease must demonstrate both non-inferiority to the first-in-class product and superiority to the current standard of care on both mortality and feeding inhibition endpoints. Experimental hut trials with free-flying mosquitoes are currently the gold-standard method for evaluating ITNs. However, due to variability in mosquito densities at field sites it may be necessary to carry out large studies to get enough power to measure the non-inferiority of ITNs. Therefore, the WHO has encouraged exploration of other potential alternative test methods including the I-ACT (Ifakara ambient chamber test). In this study, I-ACT and experimental huts were compared for non-inferiority studies. Three net products were evaluated in fully randomized, double blinded equivalence field studies to compare the performance of the investigational interventions to active comparators following WHO guidelines. Study 1: an alpha-cypermethrin + PBO LN in comparison to alpha-cypermethrin only ITN. Study 2: alpha-cypermethrin and pyriproxyfen ITN against alpha-cypermethrin only ITN. Study 3: deltamethrin incorporated polyethylene ITN compared deltamethrin-coated ITN. The nets were first evaluated in experimental huts and then the same nets were subsequently evaluated in the I-ACT using both pyrethroid susceptible and pyrethroid resistant mosquitoes. In each experimental hut study, products performed very similarly to active comparators with wide 95% confidence intervals. However, in the I-ACT where 30 mosquitoes of each strain are used each night, confidence intervals were smaller and the studies were powered to detect non-inferiority at the 10% effect difference. Using the I-ACT, after 20 nights of data collection superiority of the PBO product was seen against the CYP450 resistant mosquito strain. A difference in mosquito fertility between the susceptible and resistant strains with the pyriproxyfen net was also observed and for the pyrethroid only net the incorporated product showed superiority over the coated product after 20 washes against the pyrethroid resistant strain. The I-ACT assay is useful for measuring small effect differences between new products needed for non-inferiority studies using both insecticide susceptible and resistant strains.

Evaluation of vector control products at the CREC/LSHTM Collaborative Research Programme; a GLP certified Facility in Benin, West Africa

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The CREC/LSHTM is product evaluation facility created as a collaboration between LSHTM and Benin. The Facility generates efficacy data for vector control products at all levels of evaluation: Laboratory Phase1, Phase 2 experimental hut studies, Phase 3 durability studies and community-randomised trials. In line with new PQ/WHO guidelines regarding the generation of efficacy data for vector control products, CREC/LSHTM after 3 years of hard work became the first facility in West Africa to become GLP certified for the evaluation of vector control products. The facility has generated efficacy data for several new generation products like Interceptor G2, Royal Guard LN, Olyset Plus, Fludora Fusion, which are now being considered for large-scale field use. Several other novel products are currently under evaluation at the facility. CREC/LSHTM has extensive experience in vector control product evaluation lasting over 15 years. It is core-funded by IVCC and also works directly with product development companies seeking to generate efficacy data for their products for PQ registration. CREC/LSHTM is host of a new $6 million project funded by UNITAID through IVCC to assess through a randomised control trial the efficacy of 2 new generation dual-insecticide treated LLINs.

UP ISMC Vector Control Current and Future Work

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Abstract

With 90% of malaria case occurrence in Africa, this 'hot-spot' offers ideal conditions to study and understand the dynamics of this disease. The University of Pretoria Institute for Sustainable Malaria Control (UP ISMC) in Pretoria, South Africa, provides a multidisciplinary approach towards the generation of new knowledge on the topic of safe malaria control in Africa, through fundamental and applied research. One of three chief dedicated research themes of the UP ISMC is vector control, which an essential prerequisite for any malaria research programme. Despite the implementation of insecticide based control methods (IRS and LLNs) since the 1940s in South Africa (RSA) and neighbouring countries, malaria transmission is still occurring. It has, therefore, been recognised that the development of novel and innovative control methods is required to achieve decreased incidence and mortality rates. Current UP ISMC research focuses on continual vector surveillance in endemic malaria regions of northeastern RSA and southern Mozambique for better understanding of malaria vector ecology and behavior, towards development of novel and innovative control methods. The UP ISMC has achieved such an innovative control method, together with national and international collaborators and funded by the Deutsche Forschungsgemeinschaft (DGF), by the successful development of controlled release of volatile mosquito repellents from polymer scaffolds to lower bite rate and, therefore, reduce overall malaria. The development of novel mosquito-repellent polymer-foot wear with long lasting protection significantly reduced lower limb bites under laboratory

conditions with An. arabiensis. A footwear prototype for use in rural areas of Africa, will be trialed in 2020 in the high malaria incidence region of Chokwe, Mozambique. Future UP ISMC innovative research efforts will include chemical analysis of profiles associated with malaria mosquito communication (semiochemistry), remote sensing and education and training towards vector control in Africa.

How does the long persistence of insecticides used in public health drive insecticide resistance?

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Abstract

Insecticide levels generally decline after deployment. We recently analysed mosquito mortality data which strongly suggests that low levels of insecticides persisting from deployments months or even years earlier, can be potent drivers of insecticide resistance i.e: South, A., et al. (2019). "The role of windows of selection and windows of dominance in the evolution of insecticide resistance in human disease vectors." Evolutionary Applications. DOI: 10.1111/eva.12897. Having identified and quantified this effect, we now need to consider its likely impact as a driver of insecticide resistance in public health contexts, and how its threat can be mitigated or removed. This poster summarises the key drivers of selection, but its main aim is to solicit input from the vector control community about how this research can be extended and applied.

Behavioral interaction of mosquitoes with topical repellents: a 3D flight trajectory analysis

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Abstract

Switzerland Topical repellents are important tools for personal protection against mosquito bites and are available as lotions, sprays, gels, roll-ons, etc. that may contain a range of active ingredients. Though several active ingredients may provide protection over several hours we have little concept as to how these topical repellents impact mosquito behaviour. A better understanding of how mosquitoes interact with topical repellents may, however, lead the way to improved formulations or designs of more realistic assays for product evaluation. We measured the behaviour of host-seeking mosquitoes combining the standard arm-in-cage test with Trackit 3D, allowing for measuring the 3D flight trajectories of several mosquitoes with 90 position data points in real time. The real time functionality allowed us to monitor the measurement during the experiments and write the position data on disk without the need to store large amounts of video data, and process these off line. Filming under near-infra-red lighting allowed us to measure the natural behaviour of both day active Aedes and night active Anopheles mosquitoes under naturalistic lighting conditions. We are able to measure

the 3D flight trajectories of several mosquitoes in parallel and in real time in the vicinity of topical repellents using a novel approach, and we will present our latest results.

"Characterization of larval habitats for Anopheles funestus mosquitoes in south-eastern Tanzania"

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Abstract

In Tanzania most of malaria transmission has been contributed by Anopheles funestus, which mediates nearly nine in every ten infectious Plasmodium sporozoite infections. However, little is known regarding these mosquitoes, their ecological requirements and survival strategies. We systematically searched and characterized the aquatic habitats of An. funestus in seven villages in rural Tanzania. The study areas were surveyed for presence of water pools along 1000m transects, and the pools were labeled, georeferenced, characterized and examined for the presence of Anopheles larvae by employing standard 350mls dipper or 10L bucket. A total of 111 habitats observed in five villages, 36 (32.4%) had An. funestus larvae while 24 (21.6%) were other Anopheles species, 23 (20.7%) culicine and 28 (25.2%) had no mosquito larvae. These were mostly permanent/semi-permanent habitats with depth greater than 50cm. Habitats with stagnant/slow moving water located within 100m away from human dwellings also harbored An. funestus larvae. However, we identified surprisingly prolific habitats for An. funestus in the remained two villages, breeding along the rivers where water movement is slow and comprised with emergent kind of vegetations. The study demonstrated that, An. funestus aquatic habitats in rural Tanzania villages displaying their unique features and characteristics; they are few, fixed and findable. Larval source management targeting An. funestus can be integrated together with the primary interventions such as long-lasting insecticides nets (LLINs) and indoor residual spraying (IRS) to reduce vector population and overall malaria transmission.

"Malaria vector surveillance in elimination and post elimination phase in Iran"

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Abstract

Iran has achieved a dramatically decrease (95%) in malaria cases between 2000 and 2018, from 12,294 cases to 625 reported cases and there are no any indigenous cases DURING last two years. Iran has plan to achieve the elimination certificate BY 2025. Iran Malaria Elimination Plan (IMEP) in calls for implementation of integrated vector management approaches to ensure evidence-based decisions for optimal and rational use of resources. It also calls for vector surveillance, monitoring and evaluation of vector control interventions and for routine monitoring of susceptibility of malaria vectors to insecticides.

Receptivity and vulnerability are two important criteria to establish the vector serveillance and vector control intertvention in elimination and post elimination phase as well as foci/case classification and epidemiological stratification. According to these criteria, it will be determined SOME sentinel site to do surveillance and also it will be classified the turnover of activities that will be cost effective and also sustainable plan for prevention of re-establishment.

"ECDC's activities on vector-borne diseases"

Briët, Olivier [1]

[1] European Centre for Disease Prevention and Control

Abstract

The strategic objectives of the European Centre for Disease Prevention and Control (ECDC) on vector-borne diseases (VBDs) are to strengthen EU/EEA capacity for early detection, confirmation and surveillance of, and EU/EEA preparedness and response to these diseases, as well as to assess effects of social and environmental infectious disease determinants. Through The European Surveillance System (TESSy), ECDC reports annually on notifiable diseases, including dengue fever, chikungunya, Zika virus disease, yellow fever, Japanese encephalitis, Rift Valley fever, West Nile virus (WNV) infections, Crimean-Congo haemorrhagic fever, Lyme neuroborreliosis, tick-borne encephalitis, tularaemia, Q fever and plague. During the transmission season of WNV, infections are mapped and reported weekly. Through VectorNet, a joint project with the European Food Safety Authority, ECDC produces maps of the distribution of mosquitoes, ticks, sand flies and *Culicoides* of medical and veterinary importance in Europe, and provides technical entomological advice and trainings. ECDC also commissions the development of mathematical analyses and tools to support risk assessment of VBDs (e.g. of malaria in Greece) and the development of vector control strategies against VBDs (e.g. WNV and *Aedes*-borne diseases), as well as communication tool kits (e.g. on the prevention of chikungunya and of tick-borne diseases).

"Results of Larval Control in some African Countries Using Biolarvicides"

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Abstract

Two Biolarvicides for mosquito control based on *Bacillus thuringiensis*, var. *israelensis*, H-14, strain 266/2 and *Bac. sphaericus*, strain 2362 respectively, were used in an integrated strategy with other methods of vector control in the Malaria Control Programs of the Ministries of Health in some countries, along with the use of impregnated bednets and Indoor Residual Spraying and the consultancy of Labiofam experts, obtaining very good results.
It targets not only the diseases transmission, but also the reduction of all the species of mosquitoes present in the breeding sites to attain the well-being of the people in the communities involved, therefore can be applied also in touristic areas to diminish the mosquito´s bites in outdoors locations.
Taking into account the results of the applied programs it is presented, as has been recorded by the local health authorities, the reduction of the relative larval densities in the breeding sites and the reduction in malaria cases in Ghana, Angola, Equatorial Guinee, Burkina Faso, Gabon and Zambia.
As a conclusion of the results obtained we recommend the inclusion of Larval Source Management strategies and especially biolarviciding in the National Malaria Control Programs of the countries, mainly in those trying to achieve the elimination of this disease in 2020.

"Estimating the impact of spatial repellents and odour baited traps on malaria transmission from semi-field experiments"

Adrian Denz[1,2], *Margaret Mendi Njoroge*[3,4], *Mgeni Mohamed*[5], *Sarah Moore*[1,5], *Fredros Okumu*[5], *Ulrike Fillinger*[4], *Alexandra Hiscox*[3,4], *Adam Saddler*[1], *NakulChitnis*[1,2]

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Abstract

New mosquito control tools are needed to address residual malaria transmission. Candidate tools are often evaluated based on biting inhibition measures from semi-field studies, while their killing potential is often neglected due to difficulties in direct measurements. This can lead to wrong decisions, given the much higher epidemiological impact of mosquito removal vs diversion. We developed a statistical model that can estimate the deterring and the killing effect (before as well as after biting) of candidate tools from semi-field experiments with time-stratified human landing catch data. Using an existing model for malaria in mosquitoes, we can predict the effect on vectorial capacity of a wide variety of tools tested in semi-field experiments, including an estimate of uncertainty. With this methodology we analysed semi-field data for spatial repellents (transfluthrin-treated eave ribbons, push), odour-baited traps (Suna trap, pull) and their combination (push-pull) from studies with An. arabiensis in Kenya and Tanzania. In this setting we found the effect on vectorial capacity was strongest for push, due to its high killing potential. This work suggests that transfluthrin inhibits bites not predominantly by repelling mosquitoes as previously assumed but by substantially killing host seeking mosquitoes. This suggests that spatial repellents can be an effective tool in reducing indoor and outdoor malaria transmission.

"Trends in insecticide resistance in African Malaria Vectors"

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Abstract

Insecticide resistance in malaria vectors has the potential to derail control and impede elimination efforts. To better understand the impact of insecticide resistance, we need to quantify temporal and spatial trends. Using WHO susceptibility tests performed on 6,423 *Anopheles gambiae* s.l. samples, we quantified variation in the prevalence of insecticide resistance to pyrethroids and DDT over the period 2005-2017. Our east and west models were informed by a suite of potential explanatory variables describing the coverage of insecticide-based vector control programme interventions, agriculture and the relative abundance of sibling species.

The predictions generated by this spatio-temporal model can be used to generate tools that are potentially useful in vector control decision making. For example, we have calculated the probability for a district is below a threshold value, for a sliding scale of thresholds from 10 to 90%.

"Non-Inferiority and superiority testing of Insecticide Treated Nets (ITNs): Power simulations from experimental huts and Ifakara Ambient Chamber tests"

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Abstract

A non-inferiority trial aims to demonstrate that the test product is 'not unacceptably worse' than the comparator by more than a pre-specified, small amount. Candidate Insecticide treated nets (ITNs) are not required to provide epidemiological data for their assessment. Instead, they are assessed by the WHO Prequalification Team (PQT) based on their safety, quality and entomological efficacy data. It must be demonstrated that New ITNs are not worse than the comparator ITNs in Experimental hut trials against free-flying mosquitoes to ensure that all new products going to market are of high

protective efficacy. In order to be sure that nets truly are no different it is important to have adequate replication (sample size) because the less rigorously conducted the trial, the easier it can be to show non- inferiority due to large confidence intervals. Therefore, we conducted sample size calculations for common trial designs (non-inferiority and superiority) used for ITN evaluations. Sample sizes required to demonstrate non-inferiority with a lower margin non-inferiority of >0.7 and to demonstrate 10% superiority at 0.05 significance on the mortality endpoint were simulated a thousand times in R statistical software using a mixed effect binomial regression model. A fully randomised Latin square design of 7 x 7 arm (non-inferiority) and 5 x 5 arm study (standard experimental study) and varying mosquito densities of 5, 10, 15, 20, 25 and 30. The heterogeneity for daily observation and efficacy estimate for pyrethroid nets were set respectively at 0.45 and 0.55 for West African huts (WH), 0.64 and 0.25 for Ifakara huts (IH) and 0.9 and 0.85 for Ifakara Ambient Chamber Tests (IACT) based on a previous study. Sufficient power (≥80%) for a 7x7 non-inferiority trial was reached at geometric mean of 10 mosquitoes in WH, 15 in IH and 20 mosquitoes in IACT. Sufficient power (≥80%) for a 5x5 superiority trial was reached with 20 mosquitoes in WH and 15 mosquitoes in IH and IACT. The simulations demonstrate that both non-inferiority and superiority trials of ITNs are feasible in existing experimental sites even when the true difference between products is small.

"Better methods for LLIN specifications and characteristics"

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Abstract

Many of the existing methods used for acceptance of LLIN by WHO are based on chemical and physical assays that are not very informative for bed nets and do not consider aspects of dosage variation depending on production methods. Further, the methods are not standardized between them. IIC with partners Vegro and Biolytrics Laboratory have started developing test and sampling methods to improve baseline data used for characteristics and specifications. We here show how to minimalize inter samplevariations to obtain that nets samples representing pre- and post-treatment data (e g washings) have the same start values. We then use this samling method when determining surface concentrations and wash off, the latter as done for the regeneration timestudy, a parameter that is used in all later bioassays and chemical analysis reslting in an OK or not from WHO. The data show that 3 washes in a day does not wash off all insecticide as anticipated in the WHO defined test, but roughly the half. The work willcontinue through 2020 and hopefully lead to better specifications and net chracteristics than we have today for LLINs.