Study Protocol

TITLE: Quantifying the Epidemiological Impact of Targeted Indoor Residual Spraying on Aedes-borne Diseases

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Quantifying the Epidemiological Impact of Targeted Indoor Residual Spraying on *Aedes*-borne Diseases

Application to:
National Institute of Allergy and Infectious Diseases (NIAID)

DMID Funding Mechanism:
U01

Principal Investigator:
Gonzalo Vazquez-Prokopec, MsC, PhD

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Statement of Compliance

The study will be carried out in accordance with Good Clinical Practice (GCP) as required by the following [use applicable regulations depending on study location and sponsor requirements; samples follow]:

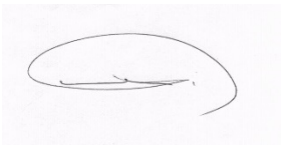
- *U.S. Code of Federal Regulations applicable to clinical studies (45 CFR 46)*
- *ICH GCP E6*
- *Completion of Human Subjects Protection Training*
- *NIH Clinical Terms of Award*

Refer to: *http://www.hhs.gov/ohrp/humansubjects/guidance/45cfr46.htm#46.*
http://www.fda.gov/cder/guidance/959fnl.pdf
http://grants.nih.gov/grants/guide/notice-files/NOT-OD-01-061.html
http://cme.cancer.gov/c01/

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Site Investigator:*



Signed: _____ Date: 01/14/2019

Name: Pablo Manrique-Saide
Title: Professor, Autonomous University of Yucatan.

Table of Contents

Table of Contents - *continued*

page

List of Abbreviations

ABV	Aedes-borne virus (ABVs, Aedes-borne viruses)
AE	Adverse Event
CFR	Code of Federal Regulations
CHIKV	Chikungunya virus
CIOMS	Council for International Organizations of Medical Sciences
CRCT	Clustered randomized controlled trial
CRF	Case Report Form
DENV	Dengue virus
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH, DHHS
DSMB	Data and Safety Monitoring Board
FSD	Familias sin dengue (cohort study)
FWA	Federal-Wide Assurance
GCP	Good Clinical Practice
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IEC	Independent or Institutional Ethics Committee
IRB	Institutional Review Board
ISM	Independent Safety Monitor
JAMA	Journal of the American Medical Association
MOH	Ministry of Health
MOP	Manual of Procedures
N	Number (typically refers to subjects)
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health
PI	Principal Investigator
SAE	Serious Adverse Event
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
TIRS	Targeted Indoor Residual Spraying
WHO	World Health Organization

Title: **Quantifying the Epidemiological Impact of Targeted Indoor Residual Spraying on *Aedes*-borne Diseases**

Population: 4,600 children aged 2-15 at enrollment, primarily Hispanic and of mestizo origin. All healthy at enrollment.

Number of Sites: *1*

Study Duration: *5 years*

Subject Duration: *4.5 years* (1 year baseline, up to 3.5 years of trial follow-up)

Objectives:

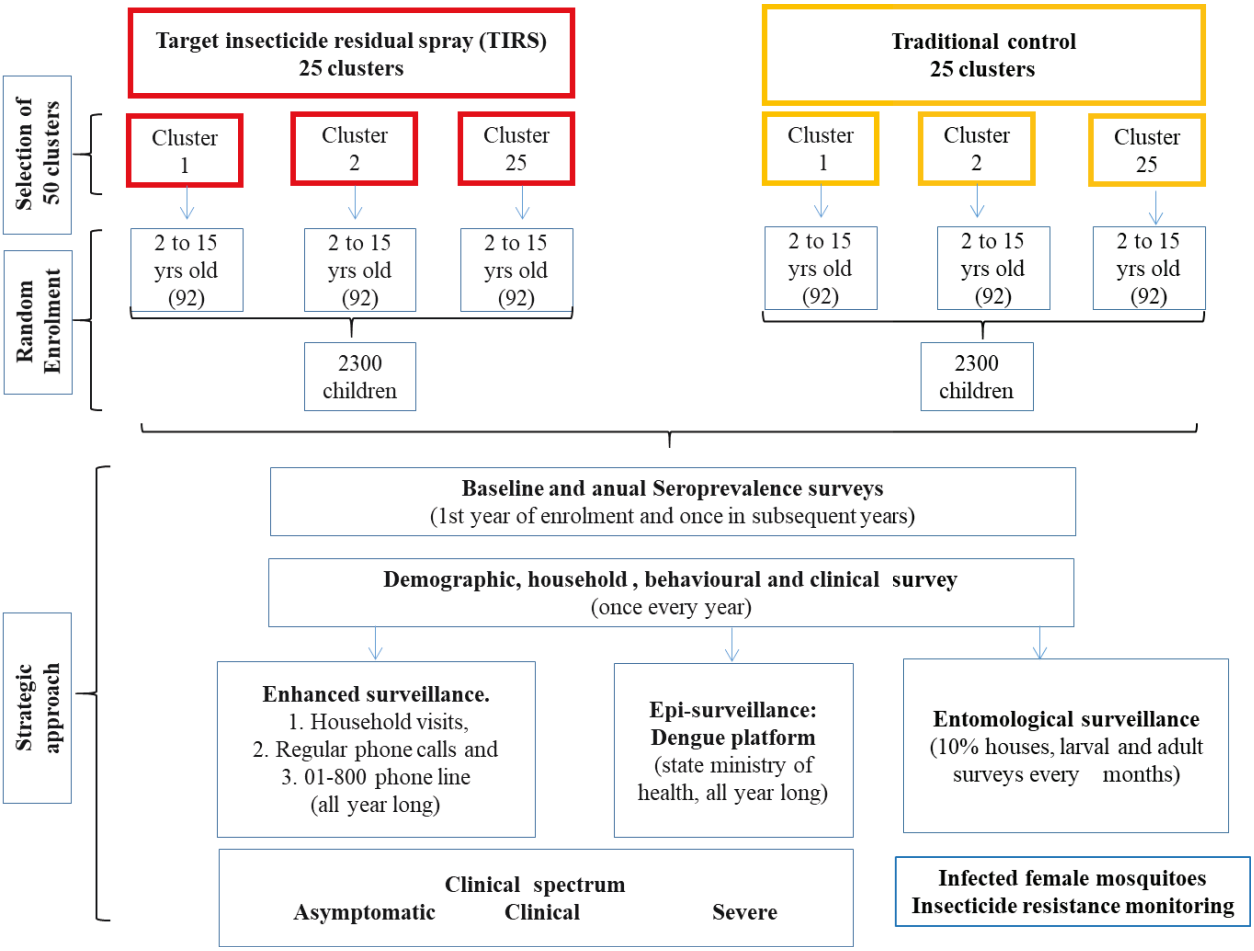
Our overall study objective is to **quantify, via a two-arm, parallel, unblinded, CRCT, the efficacy of TIRS in preventing symptomatic disease caused by ABVs in children 2 to 15 years of age at baseline from the city of Merida, Yucatan State, Mexico.**

We hypothesize that performing preemptive control with TIRS (e.g., applying insecticide with high residual activity before the onset of peak transmission season) will significantly reduce ABV burden in comparison to reactive vector control activities routinely implemented to control ABVs (truck-mounted ULV spraying, larviciding). Our proposed TIRS trial will also be highly operationally relevant in that it will quantify the impact of an approach with potential for rapid scale-up and implementation within existing MOH infrastructures.

Primary Endpoint: Laboratory-confirmed (virologically [RT-PCR testing of acute samples] or serologically [IgM and IgG ELISA testing of paired acute and convalescent samples]) symptomatic DENV, CHIKV or ZIKV.

Secondary endpoints: **a)** Laboratory-confirmed (serologically, [IgG ELISA and neutralization testing of annual surveillance samples]) DENV, CHIKV or ZIKV infection; **b)** Ae*. aegypti* mosquito infection rates with DENV, CHIKV or ZIKV (assessed by RT-PCR); **c)** *Ae. aegypti* indoor entomological indices (adult presence and abundance, female presence and abundance, bloodfed female presence and abundance).

Schematic of Study Design:



1 KEY ROLES

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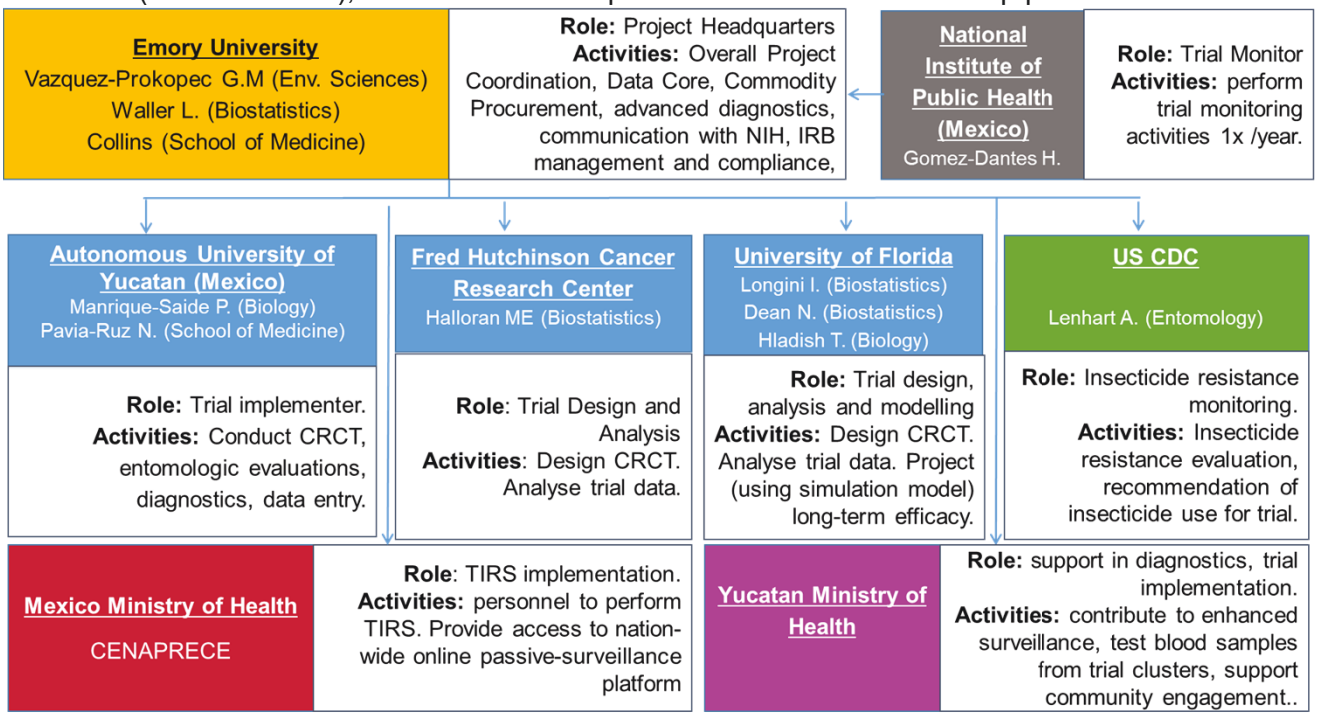
Emory will be the project headquarters. **Gonzalo Vazquez-Prokopec, MSc;PhD** will be the project PI and will be in charge of overall project coordination, sub-award management communication with NIH and ensuring that milestones are accomplished in a timely manner. **Lance Waller, PhD,** will lead the data infrastructure and management components of the proposal, which will include overseeing a full-time database manager in the development of the REDcap database schema, setting-up forms for field data collection, overseeing data entry and integrity, providing data access and support to all project members, ensure data storage, access and sharing is HIPAA compliant. **Matthew Collins, MD, PhD,** will oversee all diagnostics components of the project, both in Merida and at Emory University. Through prior funding from Emory University Global Health Institute, Dr. Collins has set-up testing platforms in Merida and in Emory University, both capable of performing similar diagnostics (PCR, ELISA IgM, IgG). Dr. Collins will be responsible of diagnostics quality control by communicating periodically with UADY lab personnel, and by performing blind-tests of a sample of serum samples at Emory University to compare validity of field results. Dr. Collins's lab is fully functional for performing all tests (e.g., ELISA and FRNT). Emory University's Clinical Trials Audit and Compliance (CTAC) department (http://www.ctac.emory.edu/) will coordinate with the project monitor (**H. Gomez-Dantes (MD, MPH),** National Institute of Public Health) all aspects concerning trial monitoring. Dr. Gomez-Dantes has more than 25 years' experience working with *Aedes*-borne viruses, including the oversight of longitudinal cohort studies, is fluent in English and Spanish, and will visit Merida once per year to check patient records, adverse events and participant drop-out records and report to the project's PI any findings. The figure below shows roles of all sub-awardees (labeled in blue), and here we will report roles of those in leadership positions.



The **Autonomous University of Yucatan (UADY)**. Will be responsible for trial implementation. **Pablo Manrique-Saide, PhD**, will coordinate all aspects of the implementation of the trial, which will involve communicating with the PI about progress or challenges in the achievement of the study milestones, coordinate all regulatory components of the project (e.g., working with local IRB, coordinating spraying activities with CENAPRECE, collaboration with Yucatan MOH), hiring field personnel, and assure all commodities are procured in time. Given the complexity and magnitude of tasks, Dr. Manrique-Saide will be seconded by **Norma Pavia-Ruz, MD,** who will help coordinate all field and laboratory personnel, making sure they perform their activities according to plan. Dr. Pavia-Ruz will be responsible for ensuring that the informed consent process is performed accordingly, and will evaluate records to make sure all enrolled participants have a corresponding consent form. Additionally, Dr. Pavia Ruz will serve as the project's point of contact for any human subjects questions or matters (as an MD, she has the credentials to discuss with participants any concerns or issues they may have). Additionally, Dr. Pavia-Ruz will be responsible of talking with participants who withdraw from the study, to make sure accurate records for participant loss are kept. Dr. Pavia-Ruz will also communicate with Dr. Collins at Emory, to ensure that diagnostics are performed at the highest level. Additionally, Dr. Manrique-Saide will coordinate shipment of specimens and samples (serum and mosquitoes) to Emory University for further testing. **Fred Hutchinson Cancer Research Center (FHCRC)**. Will be responsible of trial design and analysis. **Elizabeth (Betz) Halloran, MD; PhD; MPH,** will lead the trial design component of the trial, and have a significant role in the analysis and simulation modeling of trial data. Dr. Halloran has extensive experience designing clinical trials and analyzing trial data. For this study, Dr. Halloran will oversee the implementation of the variable-constrained randomization to identify the study clusters that will be included in the trial, and advise on the statistical methods needed to quantitatively evaluate the primary and secondary endpoints. **University of Florida** will be responsible for trial analysis and modeling. **Ira Longini, PhD,** will lead all analysis and simulation efforts related to the trial. Dr. Longini will be joined by **Natalie Dean, PhD,** who will have a primary role in data analysis. Both Longini and Dean have extensive experience analyzing trial information, including a recent Ebola ring vaccination study [REF]. Specifically, Longini and Dean will have access to primary data from our study, and will use it to quantify the metrics needed for statistical inference of the primary and secondary endpoints. **Thomas Hladish, PhD,** will use the trial results (i.e., entomological and epidemiological efficacy) to project the impact of TIRS at the city and State levels. Dr. Hladish will have full access to all trial data, as he will need information on sero-prevalence, mobility and TIRS efficacy in order to parameterize the simulation model. Technical support of the US CDC (**A. Lenhart (PhD, MPH)**) for evaluating patterns of insecticide resistance in space and time will be very valuable for the study. **Mexico's Federal MOH (CENAPRECE)** will contribute with personnel for spraying and grant access to project members to their online ABD database. The **Yucatan State MOH** will provide access to samples for laboratory testing in support of the trial's enhanced surveillance procedures, as well as help with communication about TIRS and the trial's goals.

1.1 Background Information

Aedes-borne viruses (ABVs; e.g., dengue [DENV], chikungunya [CHIKV], Zika [ZIKV]) pose a major public health burden worldwide[1, 2, 3]. Transmitted primarily by the highly anthropophilic mosquito, *Aedes aegypti*, ABVs propagate epidemically, inflicting dramatic healthcare and development costs on urban tropical environments. Model projections estimate an average of 390 million DENV infections occur per year, of which 96 million manifest clinically[4, 5]. Explosive DENV outbreaks saturate healthcare systems [6], with worldwide estimates as high as $39 billion (2010 US$) per year spent on the costs of medical care, surveillance, vector control, and lost productivity[7]. The emergence and rapid epidemic propagation of CHIKV and ZIKV (and particularly congenital Zika) have added significant burden and costs to healthcare systems[8, 9]. Given the heavy global burden of ABV illness, and in the absence of efficacious vaccines or other therapeutic options, the implementation of highly impactful vector control represents the only viable approach currently available for effective prevention[10, 11].

In most of the world, vector control methods such as larval control, source reduction and space spraying are widely used against ABVs[12, 13]. Unfortunately**, there is limited epidemiological evidence that these existing methods are adequate to prevent or contain ABV transmission** in a sustainable manner[13, 14]**.** Poorly designed evaluations, a historical lack of focus on quantifying intervention impact using epidemiological endpoints, and limited funding for large-scale randomized controlled trials with epidemiological endpoints have all contributed to the lack of rigorous, evidence-based assessments of ABV vector control interventions[10, 15]. Furthermore, the classic deployment of house-based interventions in response to clinical ABV cases has failed to account for the important contribution of out-of-home human exposure to *Ae. aegypti* and to onward dengue transmission[16] and the silent contribution of asymptomatic infections[17]. **Novel vector control approaches and intervention delivery strategies with proven and robust epidemiological evidence of their impact on ABVs are urgently needed**.

Fig 1. TIRS application on walls (above; adapted from WHO 2015) and an applicator in Merida, Mexico, performing TIRS (below).

Indoor Residual Spraying (IRS) is the use of long-lasting residual insecticides applied to the walls, eaves, and ceilings of houses or structures targeting vectors that land or rest on these surfaces[18, 19, 20]. The residual component of the application means that, for several weeks or months, the insecticide will kill mosquitoes and other insects that come into contact with treated surfaces. IRS does not directly prevent people from being bitten by mosquitoes but it reduces overall mosquito density by killing mosquitoes contacting treated surfaces. IRS is used to control malaria, Chagas and leishmaniasis[19, 20, 21].

Historical evidence has shown that, when expeditiously implemented, residual insecticide applications can significantly reduce ABVs. The elimination of *Ae. aegypti* from the Mediterranean region was attributed to IRS campaigns that were conducted for malaria eradication[21], and IRS either alone [22], or in combination with larval control[23],

contributed to the elimination of *Ae. aegypti* and DENV from (British) Guyana and the Cayman Islands, respectively. Despite this evidence, the fact that it is time consuming and dependent on specialized human resources has limited IRS widespread adoption by control programs due to the perceived challenge of scaling-up the intervention over large urban areas. Work primarily led by our team has contributed to a shift in the conceptualization and implementation of IRS to control *Ae. aegypti* in urban areas (reviewed by[14, 24]). **Our approach of adapting IRS to the urban context of *Ae. aegypti* control was based on three components: a) modifying insecticide application sites to account for *Ae. aegypti* indoor resting behavior; b) piloting novel insecticide formulations that have high residual activity and to which *Ae. aegypti* are susceptible; c) changing the intervention delivery from reactive (after detection of symptomatic cases) to preemptive (prior to peak ABV transmission season).**

In urban settings, adult *Ae. aegypti* typically rest indoors, where they feed frequently and almost exclusively on human blood[25]. Studies performed in Panama and our work in Peru and Mexico have shown that *Ae. aegypti* rest predominantly below heights of 1.5 m, mainly inside bedrooms and on surfaces made of cement, wood and cloth[26, 27, 28]. Our studies performed in experimental houses in Merida, Mexico, show that selectively applying residual insecticides below 1.5 m and on common mosquito resting surfaces provides an entomological impact similar to spraying entire walls (as performed in classic IRS), but in a fraction of the time (<18%) and insecticide volume (<30%) compared to classic IRS [29]. **We call this selective insecticide application mode 'targeted indoor residual spraying' (TIRS)**, and it includes the application of residual insecticides on exposed low walls [<1.5m], under furniture, and on dark surfaces of all house quarters with the exception of the kitchen; Fig. 1. As such, TIRS can be considered as a rational vector control approach that exploits *Ae. aegypti* resting behavior to focalize insecticide applications, thus reducing unnecessary exposure to chemicals in both applicators and household residents and also reducing the time it takes to spray a premise with no apparent loss in insecticidal efficacy[29]**.** A typical house in Merida, Mexico has a floorplan area of ~70 m^2 and can be sprayed with TIRS in an average of 9.35 min (± 0.124 min SEM; n = 571 houses)[29]. **In Cairns, Australia, we performed an observational study that found that TIRS can reduce the probability of future DENV transmission by 86-96% as compared to unsprayed premises[30] (Fig. 2).** In that study, which analyzed data from enhanced surveillance and g eocoded insecticide

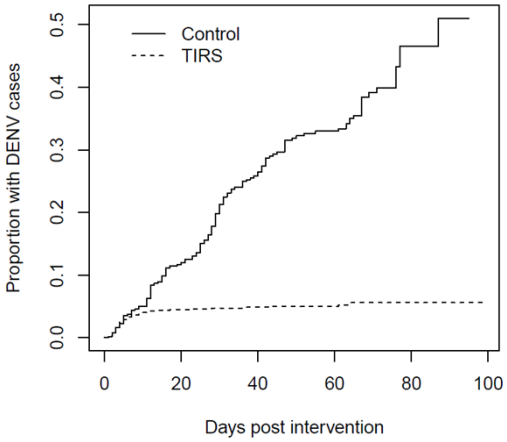


Fig 2. Epidemiological impact of TIRS on dengue in Cairns, Australia (Vazquez-Prokopec et al. 2017)**.**

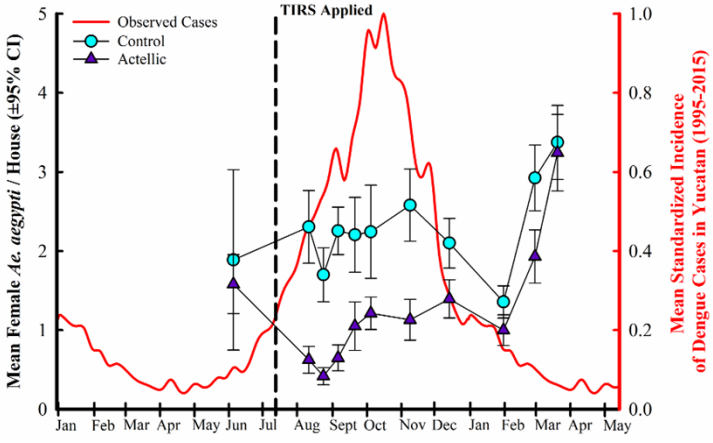


Fig 3. Entomological impact of TIRS using pirimiphos-methyl (Actellic 300CS) applied prior to the transmission season (Dunbar et al. in prep.)**.**

applications, the remarkable protection provided by TIRS led to the containment of a dengue outbreak[30], providing significant cost-savings to the local Ministry of Health (MOH)[11]. Furthermore, concurrent trap collections of *Ae. aegypti* in the heart of the outbreak showed that TIRS was associated with a ~70% reduction in female mosquito abundance[31].

As *Ae. aegypti* resistance to pyrethroid insecti cides is reported with increasing frequency[32, 33], it is crucial to select a chemical that maximizes insecticide efficacy while limiting detrimental effects to the environment or human health[34]. In Merida, Mexico, we performed a Phase II cluster randomized controlled trial (CRCT) to evaluate the entomological impact of IRS with bendiocarb (Ficam®, BAYER, a carbamate insecticide to which local *Ae. aegypti* are fully susceptible) and reported reductions in indoor adult *Ae. aegypti* abundance up to 70% over a 3-month period, compared to no reduction when the pyrethroid deltamethrin was used[34]. In further evaluations we showed that bendiocarb can yield up to 5 months of control[29]. Our most recent Phase II entomological CRCT (using the same design as in[34]) has estimated the impact of TIRS with the organophosphate pirimiphos-methyl (Actellic 300CS®, Syngenta) applied 1 month prior to the beginning of the ABV transmission season and evaluated over 8 months (Fig. 3). Actellic provided a statistically significant reduction in *Ae. aegypti* female and bloodfed female abundance for up to 7 months post-spraying, covering the entire peak transmission season (Fig. 3). Average entomologic reduction in *Ae. aegypti* female abundance (compared to control clusters) across the transmission season was 74%, similar to previous TIRS efficacy estimates from Australia[31] and Mexico[34]. **Our Phase II CRCTs and experimental studies indicate that a ~10-14 min application can reduce *Ae. aegypti* indoors by ~70% for up to 5-7 months, creating the possibility for preemptive vector control (Fig 3).**

In Yucatan, as is often observed in tropical settings, ABV transmission is seasonal, with ~84% of DENV cases reported in 1995-2015 occurring between July 1 and Dec 31 (Fig 3 and[35]). Using a stochastic simulation model fitted to Yucatan State, Mexico, we found that high levels of TIRS coverage (75% houses treated once per year) applied preemptively before the typical dengue season (before July) could reduce DENV infections by 89.7% (median of 1,000 simulations; interquartile range [IQR]:[83.0%, 94.8%]) in year one and 78.2% (IQR: [71.2%, 88.0%]) cumulatively over the first five years of an annual program [35]. Effectiveness can be increased and intervention start date pushed earlier if using an insecticide with longer residual power (~150 days, equivalent to Actellic® 300CS) than bendiocarb (~90 days)[35]. As such, our theoretical exercise shows that **conducting preemptive TIRS will provide high short-term and long-term effectiveness in preventing ABVs in areas where transmission is seasonal.**

1.2 Scientific Rationale

The only epidemiologic evidence of TIRS effectiveness comes from our work in Australia[30, 34], which challenges the applicability of such findings to highly endemic tropical settings. **Furthermore, there is no randomized evidence quantifying the impact of TIRS on clinical disease.** For TIRS to obtain a full WHO policy recommendation, information about the intervention's public health impact derived from well-designed clinical trials must be available[15]. **As such, there is a need for rigorously implemented clinical trials evaluating TIRS within ABV endemic settings**[10, 13]. Such trials will contribute to the body of evidence for the use of this intervention to prevent ABVs.

1.3 Potential Risks and Benefits

1.3.1 Potential Risks

Overall, the risks to study participants are minimal in all of our study procedures (summarized in Table 1). The most sensitive risk is related to potential intoxication with the insecticides used in TIRS.

Both Actellic 300CS® (Syngenta) and Ficam® (Bayer) have been approved by the World Health Organization (WHO) for indoor control of mosquitoes[18,19]. WHO's hazard assessments concluded that, when used for indoor residual spraying as instructed and at the recommended doses, both bendiocarb and pirimiphos-methyl 300 CS do not pose undue hazards to the spray operators or residents of the treated dwellings[36, 37]. Provided that operational guidelines are followed, routine cholinesterase monitoring of spraymen during indoor residual spraying programs is not required[36, 37].

Both pirimiphos-methyl and bendiocarb insecticides, are reversible inhibitors of cholinesterase, an essential nervous system enzyme. Pirimiphos-methyl can cause cholinesterase inhibition in humans; that is, it can overstimulate the nervous system causing nausea, dizziness, confusion. Very high exposures (e.g., accidents or major spills) to pirimiphos-methyl can produce respiratory paralysis & death[38]. Organophosphate vapors rapidly produce mucous membrane and upper airway irritation and bronchospasm, followed by systemic muscarinic, nicotinic and central effects if exposed to significant concentrations[38]. Symptoms of bendiocarb poisoning include weakness, blurred vision, headache, nausea, abdominal cramps, chest discomfort, constriction of pupils, sweating, muscle tremors, and decreased pulse[39]. If there is severe poisoning (e.g., accidents or major spills), symptoms of twitching, giddiness, confusion, muscle incoordination, slurred speech, low blood pressure, heart irregularities, and loss of reflexes may also be experienced. Death can result from discontinued breathing, paralysis of muscles of the respiratory system, intense constriction of the openings of the lung, or all three [39]. Carbamates generally do not accumulate in mammalian tissue and the cholinesterase inhibition reverses rapidly once exposure ceases. Complete recovery from an acute poisoning by bendiocarb, with no long term health effects, is possible if exposure ceases and the patient has time to recover their normal level of cholinesterase before succumbing to symptoms. In non-fatal cases, the illness usually lasts less than 24 hours. Bendiocarb vapors rapidly produce mucous membrane and upper airway irritation and bronchospasm, followed by systemic muscarinic, nicotinic, and central effects if exposed to significant concentrations [39].

Our project will carefully monitor potential exposure to chemicals on our study population. In our Phase II entomological trial utilizing Actellic 300CS, we performed individual acceptability surveys in 160 houses, including 630 individuals (Vazquez-Prokopec et al. unpublished). Our survey detected 19 cases (3%) of symptoms compatible with a reaction to the insecticide. The most common signs (accounting for 85% of symptoms) were headache, nausea and skin irritation. All of the symptoms reversed within 1-2 days without the need for medical intervention. Our study will treat any signs of intoxication as adverse events and will report them accordingly (see DSMP section). As for insecticide applicators, we will follow WHO recommended practices, which include wearing personal protection equipment and making insecticide dilutions using pre-filled sachets with the right amount for each machine charge (see manual of procedures for detailed steps).

Agricultural use of bendiocarb and pirimiphos-methyl have been associated with risk of environmental contamination[38, 39], as both insecticides are soluble in water and toxic to birds, fish and arthropods. The doses and mode of application in indoor residual spraying are not seen as a source of environmental contamination by WHO, due to difficulty in having insecticides runoff into surface waters. There is a risk of environmental contamination if water used for washing spraying equipment is not properly handled. We will follow Mexico's MOH strict procedures for insecticide preparation, equipment cleaning and insecticide disposal (http://www.cenaprece.salud.gob.mx/programas/interior/vectores/descargas/pdf/guia_rociado_residual_intradomiciliar.pdf).

Risks associated with venipuncture include occasional bruising and a slight risk of infection at the site of the venipuncture and, in a small number of participants, dizziness associated with a vasovagal response. We do not anticipate any risks associated with our active surveillance. The information from this study is not expected to lead to any negative ramifications such as legal or employment risks, nor is there is a possibility of physical, psychological, social or legal injury from participation in this study and standard measures to protect confidentiality should be sufficient. Movement surveys collect detailed information about specific whereabouts of individuals prior to infection (Retrospective Movement Survey) or when healthy (Prospective Movement Survey), but their sensitivity and risk are also minimal.

Study Component	Risks
Intoxication due to unintended exposure to insecticides.	Direct (contact) or indirect (inhalation of fumes) intoxications are rare but likely. Bendiocarb: Symptoms of poisoning include excessive sweating, headache, chest tightness, giddiness, nausea, vomiting, stomach pains, salivation, blurred vision, slurred speech and muscle twitching. Pirimiphos-methyl: can cause cholinesterase inhibition in humans; that is, it can overstimulate the nervous system causing nausea, dizziness, confusion.
Febrile surveillance Longitudinal cohort	Pain or discomfort, bruising, or infection at venipuncture site or temporary dizziness during blood draw. Use of identifiable information (demographic information, address, febrile status)
DENV+, CHIKV+, ZIKV+ participants	Same as for febrile surveillance and longitudinal cohort The data gathered in this project will be identifiable and certain data types, such as movement interview are sensitive. The primary risks lie with identifying the individuals who provided information they consider confidential (e.g., movement to private locations). There is a small risk that the repeated blood collections will cause or exacerbate anemia.
In-depth interviews (prospective and retrospective movement interviews)	Risks to study participants are minimal. Participants may feel that in-depth interviews take up too much time – but they have the option of ending their participation at any time. There are no sensitive topics covered, but if any participant feels that there is something he/she does not want to talk about, he/she does not need to answer all questions.

Table 1. Potential risks associated with specific components of our study.

1.3.2 Known Potential Benefits

Our experience with Phase II trials of TIRS has shown that households receiving the intervention see a measurable reduction in the number of *Ae. aegypti* as well as other nuisance insects. For instance, *Culex quinquefasciatus* mosquitoes are highly abundant indoors, and householders report being bitten significantly less at night (when this vector is active). Other perceived benefits of the intervention include the fact that cockroaches, spiders and scorpions are also killed by residual insecticides. Householders report seeing dead insects as a sign of the quality and efficacy of the insecticides applied. In fact, a survey performed on 160 households receiving TIRS with Actellic 300CS reported that 96% of house owners would recommend the intervention to others or would receive it again, if provided (Vazquez-Prokopec et al. unpublished).

For viremic participants captured in active surveillance, the clinical laboratory testing and medical consultation will allow detecting severe cases that may require hospitalization. A common problem in Merida, like other resource-poor environments, is that residents hesitate to seek medical help because of economic constraints or fear of hospitals, and allow their disease to progress to a stage where clinical intervention is unsuccessful. Our staff can reassure and advise participants to seek care, help navigate the hospital admission process, and provide evidence of dengue infection to local physicians. In practice, a definitive diagnosis of dengue infection may be useful to aid local physicians in justifying a more aggressive clinical approach for dengue-infected patients. Our enhanced surveillance, which will test samples that otherwise do not get tested at the state laboratories, will provide benefits to the public health care system, as it will enhance quantifications of magnitude of disease throughout the transmission season.

Entomological surveys alert households to *Ae. aegypti* production sites and our staff can explain how production sites can be removed. We also typically remove a significant portion of the adult female mosquitoes residing in the household in our routine collections. Although this is a temporary measure, it can provide some relief from biting insects.

2 OBJECTIVES

Our overall study objective is to **quantify, via a two-arm, parallel, unblinded, CRCT, the efficacy of TIRS in preventing symptomatic disease caused by ABVs in children 2 to 15 years of age at baseline from the city of Merida, Yucatan State, Mexico. We hypothesize that performing preemptive control with TIRS (e.g., applying insecticide with high residual activity before the onset of peak transmission season) will significantly reduce ABV burden in comparison to reactive vector control activities routinely implemented to control ABVs (truck-mounted ULV spraying, larviciding).** Our proposed TIRS trial will also be highly operationally relevant in that it will quantify the impact of an approach with potential for rapid scale-up and implementation within existing MOH infrastructures.

3 STUDY DESIGN

We will pursue a two-arm CRCT with a total of 50 clusters of 5x5 city blocks each, including at least 92 enrolled age-eligible participants per cluster, with 25 clusters randomly allocated to receive TIRS and 25 clusters not receiving TIRS (Fig 5) (see *section 4.4 in the Clinical Trial Protocol*)**. A total of 4,600 age-eligible individuals will be recruited for the trial. This sample size accounts for a 20% loss to follow-up.** Routine MOH vector control actions (primarily, truck-mounted ULV spraying of chlorpyriphos, larviciding with altosid® and indoor space spraying in premises of suspected ABV cases) performed in response to symptomatic ABV cases reported to the healthcare system will not be interrupted and could occur across both study arms. Therefore, **our trial will compare TIRS to routine ABV prevention and control, providing an opportunity to quantify intervention efficacy and potential benefits in comparison to existing practice**. Clusters will be located within the areas identified as hot-spots of ABV transmission[40] (Fig. 5). **Placing all clusters within the zone of high ABV incidence will increase power by focusing on an area with higher event rates and lead to a more homogeneous design (e.g., less likely to have imbalance across trial arms).** We will use a covariate-constrained randomization strategy[41] to randomly select 50 clusters from the set of census tracts in the hot-spot region. This approach ensures that key cluster-level variables (e.g., historical ABV transmission, population size and density, % employed population) are not more than 10% different between trial arms and maximizes the distance between neighboring clusters. **To further reduce contamination and edge effects, while all households in TIRS clusters will be offered the intervention, epidemiological and entomological evaluation will occur in the center of each cluster, following a "fried egg" design (blue blocks in inset of Fig. 5).** Enrollment into the trial will occur in all clusters before TIRS allocation has been determined, in order to prevent selection bias.

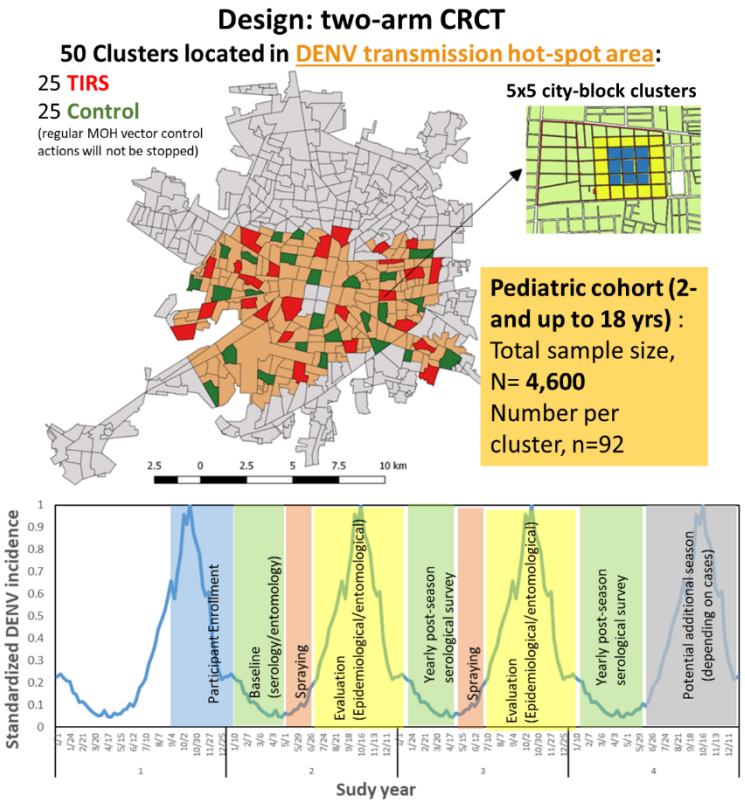


Fig 5. Proposed trial design. City blocks in yellow show the size of clusters within census areas, and blue blocks show the area where epidemiological and entomological evaluations will be concentrated ("fried egg" design).

The steps involved in the epidemiological evaluation of TIRS, in order, are (Fig 5): **a)** random selection of 50 clusters into two groups (TIRS allocation not yet determined); **b)** participant enrollment and consent/assent to participate in the trial; **c)** baseline ABV serological and entomological surveys immediately following participant enrollment; **d)** random allocation of one group to TIRS; **e)** implementation of

TIRS intervention; **f)** active case detection and monthly entomological collections post-intervention and for a period of 6 months after spraying; **g)** a post-season serological survey in all enrolled children. Steps e), f), and g) will be performed for two transmission seasons, with the potential to add a third season should incidence of the primary endpoint be lower than assumed (Fig 5). Each step will be explained in the sub-sections below. **See table below for a timeline**.

Community Involvement: Engaging communities early in the trial will be essential for maximizing participant acceptance and retention[42, 43]. We will continue involving our experienced team of 10 social scientists and social workers, who interact directly with study participants (through informal conversations, games and other educational activities with children) to ensure they remain engaged throughout the duration of the study [43]. Other factors previously highlighted as important by the communities are: **a)** the involvement of MOH personnel in spraying, rather than personnel specifically contracted for the trial; **b)** the availability of physicians who will provide direct care to enrolled children and their families, including medical diagnoses and results of laboratory tests; **c)** the participation of the local university, the Autonomous University of Yucatan.
3.2.8. Baseline study: Following participant enrollment, we will conduct a baseline epidemiological and entomological assessment (preceding the first TIRS application) to assess household characteristics (size, building materials, number of rooms, number of inhabitants), quantify levels of ABV baseline seroprevalence in enrolled children, and measure *Ae. aegypti* infestation and susceptibility to insecticides (Fig 6). All enrolled children will provide a blood sample by venipuncture, which will be tested for the presence of antibodies against DENV, CHIKV or ZIKV. A proportion (10%) of all houses located in the center of the cluster (blue blocks in Fig 5, equal to 1,350 houses across 50 clusters) will be visited monthly to collect mosquitoes indoors and outdoors, which will be used to quantify patterns of *Ae. aegypti* abundance and collect eggs that can be reared for assays to detect insecticide resistance.

Intervention: Personnel from CENAPRECE with experience performing IRS will conduct the spraying after being trained using our study procedures. Based on our model predictions[35], spraying should start May-June and extend for 1-2 months. *Ae. aegypti* in Merida and Yucatan are susceptible to both carbamates and organophosphates [32]. We will prioritize the use of Actellic 300CS®, given its longer residual power in comparison to Ficam® (7-8 months versus 4-5 months, respectively). Insecticide application will follow strict SOPs developed by our team as part of a previous CDC-Emory-UADY collaboration (see manual of procedures). Residents will be asked to temporarily leave the house during treatment and wait 0.5-1 h for the product to dry before re-entering the house. Staff will wear branded uniforms with identification and use appropriate personal protective equipment (Fig 1).

Intervention evaluation. The epidemiological impact of TIRS on the primary endpoint will be evaluated by active (enhanced) surveillance to detect and lab-confirm symptomatic DENV, CHIKV or ZIKV from July 1st to December 31st of each season (the period of peak ABV transmission, Fig 3, and right after TIRS application) (see timeline). As ABV transmission in Merida is highly seasonal, and a single TIRS application covers most of the peak transmission season (Fig 3), focusing active surveillance during a 6-month period after spraying will be more efficient and cost-effective to quantify the epidemiological impact of TIRS than spraying twice and maintaining active surveillance during the period of low transmission. Epidemiological impact will be further assessed in a secondary endpoint using yearly serosurveys performed after the peak transmission season (January-April) to serologically detect interval ABV infections. Entomological impact will be measured by standardized collections of adult and immature *Ae. aegypti* in a sample of houses from each cluster during July 1st-December 31st, coinciding with active surveillance.

Enhanced ABV symptomatic case detection for the primary endpoint will rely on three sources: 1) community-based active surveillance based on wellness visits (1x/week) or regular phone calls (2x/week) by nurses**; 2)** self-reported illness by study participants to a toll-free 01-800 number; **3)** State of Yucatan passive surveillance of ABV cases (investigators will mine these data to capture symptomatic ABV cases not identified from source 1 or 2 above). **Ten field teams consisting of a nurse and a social worker or anthropologist each will conduct wellness visits to all enrolled children once per week, with the goal of identifying any probable ABV case.** In addition to wellness visits, nurses will call parent/guardians of enrolled children regularly (twice per week) to check for the occurrence of any ABV symptoms. When interacting with participant mothers, nurses will also remind them that they can call our toll-free 0-1800 number in case of any illness compatible with an ABV infection. **Widely used in our FSD cohort, the 01-800 hotline enhances the detection of symptomatic individuals by providing study participants 24-7 access to a toll-free phone number to consult an 'on call' physician about any symptom in their families** [44]. For instance, between weeks 30-50 of 2015, 373 telephone calls were received, with 32.5% of them not related to a febrile episode suggestive of dengue. Around 40% (n=149) of febrile patients that contacted the dengue line had previously consulted a physician who gave the diagnosis of probable dengue. People then requested that our study team draw a blood sample for confirmation of clinical diagnosis. **Additionally, our project will partner with CENAPRECE and the State Laboratory (see letters of collaboration) to enhance the detection and confirmation of symptomatic ABV cases from our cohort reporting to the healthcare system**. Access to the online ABV database managed by CENAPRECE [45] will allow our team to visualize the location of all suspected cases residing within our study clusters in real-time. **By mapping the geographic location of cases, we will be able to identify if enrolled households had cases not detected by our active surveillance. CENAPRECE database will also be used to map regular vector control actions performed by the MOH.**

A suspected ABV symptomatic case will be a participant with acute onset of fever (axillary temperature ≥38°C) OR a non-focal rash PLUS any one supporting symptom such as headache, conjunctivitis, arthralgia or myalgia). When a suspected ABV case meeting the case definition is identified through active surveillance, they will be visited preferably on the same day by one of our four project physicians to perform a physical examination (physical exam, temperature, vital signs). The doctor will be joined by one field team, which will obtain demographic, behavioral data, and collect blood specimens. Acute and convalescent (preferably 14-21-days later, but up to 28 days) blood specimens will be collected on each suspected case to confirm ABV infection. We will follow detailed protocols for ascertaining each case (see section 3.3.5 below). Additional information collected from each suspected ABV case at the first visit will include: demographic information (age, sex) and history of movement (by a retrospective movement survey). Once laboratory confirmation is obtained, participants will meet with our study physicians, who will explain the diagnosis and advise on potential steps if symptoms worsen. **Yearly blood samples from all enrolled participants will be collected after the regular transmission season (from January to April) to test for serologic evidence of interval infection by DENV, CHIKV or ZIKV.** We will follow the same procedures for contacting participants and obtaining blood samples as reported in our FSD cohort study [44, 46, 47], with the modification that, in addition to blood specimens, our team will perform prospective movement surveys to characterize the routine mobility patterns of participants.

The assessment of the entomological impact of TIRS will rely on monthly household-level information characterizing adult *Ae aegypti* abundance and ABV infection. Briefly, a random sample of 10% of the houses located in the center ('yolk' of our fried-egg design) of each cluster (~1,350 houses in total) will be visited and surveyed for the presence of adult *Ae. aegypti* mosquitoes indoors as described in[34, 48]. Female *Ae. aegypti* collected indoors will be pooled by city block and tested for the presence of DENV, CHIKV or ZIKV infection. Entomological surveys will begin right after TIRS spraying (July 1st), and will be performed monthly for six months (until Dec 31st). Monthly WHO cone bioassays[34, 49] will be done in a random sample of 20 treated houses and 5 untreated houses (controls) to characterize the residual efficacy of the insecticide used.

Timeline

Our trial timeline (Table belos) will extend for five years and consist of: **a)** random selection of 50 clusters into two groups (TIRS allocation not yet determined); **b)** participant enrollment and consent/assent to participate in the trial; **c)** baseline ABV serological and entomological surveys immediately following participant enrollment; **d)** random allocation of one group to TIRS; **e)** implementation of TIRS intervention; **f)** active case detection and monthly entomological collections post-intervention and for a period of 6 months after spraying; **g)** a post-season serological survey in all enrolled children. Steps e), f), and g) will be performed for two transmission seasons, with the potential to add a third season should incidence of the primary endpoint be lower than assumed. Final analysis for quantifying primary and secondary endpoints will occur after the second transmission season ends (Q3 of year 4) and continue throughout year 5 of the project.

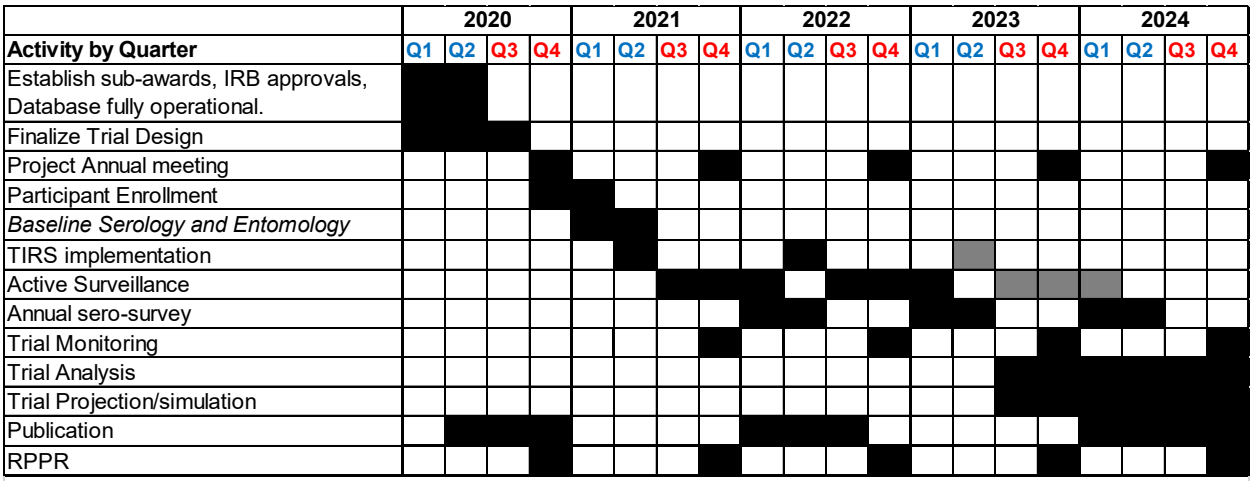


Table. Detailed timeline for milestones of the project. Red indicates quarters of high ABD transmission, whereas blue indicates quarters of low ABD transmission.

The first two quarters will be dedicated to establish the infrastructure and regulatory components of the trial. Emory University will lead all administrative components, which will include centralizing IRB protocols and approvals (in Mexico), establish sub-contracts with UFL, UADY and FHCRC; purchase insecticides, reagents and purchase travel for project members, coordinate with CENAPRECE all components of the spraying. Additionally, Emory University will establish the database schema and infrastructure, and provide database access to all project

members. Within the first two quarters we will produce our final trial design by refining our variable constrained randomization and making sure all clusters are eligible (for instance, they do not occur in areas that are unsafe to our personnel). Once clusters are identified, participant enrollment will begin. We estimate it will take us approximately 4-6 months to enroll the 4,600 children in the cohort. Right as enrollment occurs, and after the ABV transmission season (after December), we will conduct baseline serological and entomological survey to assess the natural immunity to ABVs in the study population. TIRS will be implemented in June-July of years 2 and 3 of the project (and year 4 if a third season is needed). Epidemiological and entomological monitoring of the trial will occur annually. After the regular transmission season we will conduct yearly blood draws to quantify seroconversions to ABVs. Once a year (right after the ABV transmission season) the trial monitor will travel to Merida to evaluate progress and identify any potential issues. Analysis and modeling of trial data will begin after the second transmission season (year 4 of the project). We will have in-person project meetings once per year in Merida and virtual meetings (using Emory's site-license for the Zoom videoconferencing system) once per month.

4 Study Population

4.1 Selection of the Study Population

Our trial will focus on the pediatric population. **Specifically, children 2-15 years of age at the time of enrollment living within the assigned clusters in the city of Merida, Yucatan, Mexico.** Briefly, the FSD cohort indicated that the majority of dengue naïve infections and seroconversions were observed in children <15 years old [47,49,50] (Fig 6) and, while they had lower symptomatic illness than adults, the chances of detecting both infection and disease is expected to be higher than older age groups due to their lower natural immunity to ABVs. CHIKV and ZIKV incidence during the first wave of their invasion into the Yucatan was also larger in the <15 year-old group (Table 2), supporting our age-group eligibility criteria. We will exclude younger children (<2 years) because of the inherent difficulties obtaining blood from small children, and potential for cross-reactivity of maternal antibodies[63].

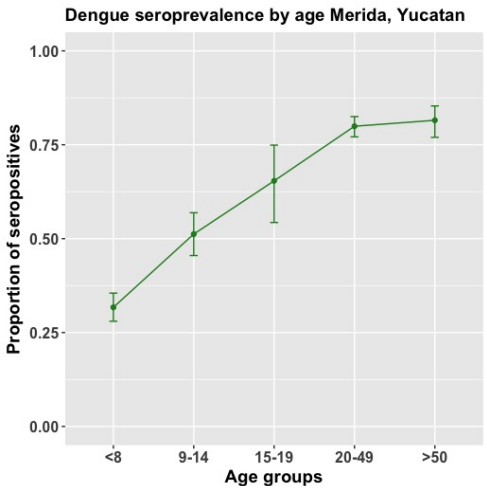


Fig. 6. Age-specific baseline dengue seroprevalence in Merida cohort, Yucatan, Mexico. From [Rojas et al.

	Person-years at risk (N)	Dengue	IR (95%CI)	Chikungunya	IR (95%CI)	Zika	IR (95%CI)
All participants	3430.9 (3400)	12	3.5 (1.9, 5.9)	30	8.6 (5.8, 12.3)	8	2.3 (0.9, 4.5)
Age groups (years)							
≤ 8	1001.4 (991)	3	2.9 (0.6, 8.8)	10	9.9 (4.8, 18.4)	4	3.99 (1.1, 10.2)
9–14	502.5 (484)	1	1.9 (0.03, 11.1)	4	7.9 (2.1, 20.4)	2	3.9 (0.5, 14.4)
15–19	145.3 (146)	1	6.88 (0.1, 38.3)	1	6.9 (0.1, 38.3)	0	0)
20–49	1490.1 (1456)	7	4.7 (1.9, 9.7)	11	7.4 (3.7, 13.2)	2	1.3 (0.2, 4.9)
≥ 50	291.5 (323)	0	0	4	13.7 (3.7, 35.1)	0	0
Gender							
Male	1511.7 (1531)	0	0	9	5.9 (5.8, 12.3)	3	1.9 (0.4, 5.8)
Female	1919.2 (1869)	12	6.3 (3.2,10.9)	21	10.9 (6.8, 16.7)	5	2.6 (0.8, 6.1)

Table 2. Incidence rates (IR) per 1,000 person-years of arbovirus confirmed symptomatic infections in the cohort in Yucatan, Mexico by age, gender. Extracted from Rojas et al. 2018[52].

There will be no exclusion criteria related to gender, ethnic or racial category. While females had slightly higher rates of ABV incidence than males (Table 2), our trial will enroll both male and female children. The health status of this population at enrollment will be mostly healthy. Based on our FSD cohort studies, we expect to enroll 50% females, and Mexican citizens, Spanish speakers, and of mestizo ethnicity (mixed Mayan and white/European). The health status of this population at enrollment will be mostly healthy.

While our study will include participants of both sexes, our analysis plan will not use such information to assess the primary or secondary endpoints and quantify the epidemiologic impact of TIRS. We will recruit 4,600 children aged 2-15. Our team has vast experience working with children as part of our FSD cohort study [47,49,50]. Our team of four physicians will include an

infectious disease pediatrician (Adolfo Palmachan, MD), who has extensive experience (~30 years) diagnosing infections locally circulating in Merida, including ABVs. Table 3 shows the sample size and procedures that will be followed for each segment of the population.

Study Component	Target N (expected or planned)	Activities
Febrile Surveillance	4,600	Census, household visits 1x/wk, phone calls 2x/wk. for two transmission seasons (and potentially 3).
Probable acute ABV illness. (From active surveillance)	1,000	2 blood samples (acute and convalescent, 14-30 d after acute) to all cases meeting the ABV case definition. Demographic and movement interviews at first visit.
Serosurvey	4,600	Provide a blood sample 1/yr for two years (and potentially 3) to quantify ABV exposure.
Loss to follow-up interviews	920	Interview participants leaving the study to assess potential causes.

Table 3. Target sample size of children aged 2-15 at the time of enrollment, and activities performed on them.

4.2 Inclusion/Exclusion Criteria

There will be two levels of participation in the trial: 1) at the household level, owners will consent to have their house sprayed with TIRS; 2) at the individual level, children 2-15 years of age at the time of enrollment will consent/assent to participate in the study, which will require their prospective follow-up for signs of ABD illness and yearly blood draws to assess seroconversion. The inclusion/exclusion criteria for each level is as follows:

1. Household Level

1.1. Inclusion Criteria:

- Household is located within the bounds of a study cluster (5x5 city-block clusters).
- City block has at least 60% premises that are residential.

1.2. Exclusion Criteria:

- Households where study personnel identify a security risk (i.e., site where drugs are sold, residents are always drunk or hostile)
- Sites where no residents spend time during the day (i.e. work 7d a week outside the home).Inability for a resident to provide informed consent.
- Non-residential places (e.g., businesses, schools, markets, etc.).

2. Individual Level

2.1. Inclusion Criteria:

- 2 or more and up to 15 years of age at the time of initial enrollment
- Living in a house that consented to TIRS.

2.2. Exclusion Criteria:

- Less than 2 years of age or more than 15 years of age at the time of enrollment.
- Not living in a house that consented to TIRS.

- Having a medical condition that prevents implementation of study procedures.
- Temporary visitor to household
- Plans to leave study area within next 12 months

5 STUDY PROCEDURES/EVALUATIONS

5.1 Study Procedures

See manual of procedures attachment for detailed procedures and forms for each component.

Intervention: Personnel from CENAPRECE with experience performing indoor residual spraying and trained to apply TIRS will conduct the spraying which --based on our model predictions-- should start between May 1st and June 1st and extend for 1.0-1.5 months. *Ae. aegypti* in Merida and Yucatan are susceptible to both carbamates and organophosphates. Either Ficam® (Bayer, a carbamate) or Actellic 300CS® (Syngenta, an organophosphate) will be used on a given year to treat all houses. We will prioritize the use of Actellic 300CS®, given its longer residual power in comparison to Ficam® (7-8 months versus 4-5 months, respectively). However, if insecticide resistance profiles of mosquitoes at baseline or after the first year of spraying show decreases in susceptibility to Actellic 300CS®, we will switch to Ficam®. Insecticide application will follow strict SOPs developed by our team as part of a CDC-Emory-UADY collaboration (see Manual of Procedures). Each spraying team will include 2 spraymen and one supervisor in charge of communicating with the household owners and evaluating the quality of spraying. Team leaders will ask the homeowner if there are areas or rooms they do not wish to have treated (and take note in the field spraying form). Residents will be asked to temporally leave the room during treatment and wait at least 30 minutes for the product to dry before re-entering the home. We will avoid treating near pets, especially near fish tanks, and do not spray children's toys, food, kitchens or exposed cloths and bedding. Staff will wear branded uniforms with identification at all times and use appropriate personal protective equipment (Fig. 1 in Research Strategy document). Relevant treatment data will be captured in the field using smartphones connected to the project's REDCap database.

Blood Samples: Venipuncture procedures will be performed using standard aseptic techniques. An experienced phlebotomist will take the blood sample from an antecubital vein. Blood volumes will depend on the participant's age (5 ml for children under 8 years and 10 ml for children 9-18 years). Blood will be collected into Vacutainer® collection tubes or by a needle and syringe. A 22-gauge needle will be used for 5-15 yr olds, and a 23-gauge needle for children <5 yrs. Serum will be separated and stored at -70°C in labeled polypropylene cryogenic vials. When results are available they will be provided to individual study participants with an explanation of their significance. We have found in previous studies that participants are curious about their results even though results often have no impact on treatment.

Entomological Surveys: *Aedes aegypti* surveys will be carried out once per month and for a period of 6 months post-TIRS application in a random sample of 10% of the households participating in the trial (equaling to ~1,600 houses per sampling period). To maintain quality control, an entomological supervisor will accompany the survey team and a second supervisor will review data daily. Adult captures: Adult *Ae. aegypti* will be collected with Prokopack® aspirators 26 and used to calculate six adult indices: prevalence and abundance of adults, females and bloodfed females.

Movement surveys: Two movement surveys will be implemented (see Manual of Procedures). The retrospective movement survey (RMS) obtains information about locations visited in the past 15 days. The RMS gathers information on the main activity performed in visited locations and the self-reported duration of the visits (frequency and time of visits). The RMS will be used to quantify the proportion of time a symptomatic individual spent within their respective cluster and identify potential contamination in the trial by pinpointing to participants who live in one cluster type (e.g., TIRS) and visit other cluster type (e.g., control). We will apply prospective movement surveys (PMS) to all subjects at the time of the yearly serosurveillance blood samples. These surveys ask questions about the routine movements, rather than prior 15 days, and will be used to quantify the cumulative time under treatment for each child. For children up to 8 years, parents are interviewed to ask about time in home and specific places visited, whereas children 9 and up are interviewed directly by our skilled team of anthropologists. The project PI, GVP, has over 10 years' experience applying and analyzing RMS and PMS instruments.

5.2 Laboratory Evaluations

5.2.1 Laboratory Evaluations/Assays

Laboratory testing will be shared between UADY and Emory as described below.

Active (enhanced) surveillance: acute samples from suspected ABV meeting our case definition will be tested at UADY using molecular testing by multiplex reverse transcriptase-polymerase chain reaction (RT-PCR) [50], NS1 detection and virus-specific IgM testing. Acute and convalescent specimens will be shared in parallel with Emory for assessment of IgM and IgG seroconversion. Testing of acute specimens in real time on site at UADY is critical as it allows immediate feedback to be given to subjects and/or their medical providers which may impact their clinical care.

Annual serologic surveillance for total ABV infection will be accomplished by obtaining a single serologic specimen from each subject each year of the study, including a baseline specimen prior to commencement of the TIRS intervention and following the final transmission season of the study (Fig 5). Testing will be performed at Emory University by a stepwise testing approach. Each ABV is considered independently – for serosurveillance, each serotype of DENV is considered as a distinct virus. Once an infection is detected, no further testing for infection by that virus is pursued in subsequent study years. Antigen capture ELISA for human IgG[51] will be the first test. Conversion from negative to positive for IgG indicates interval infection. Measurement of neutralizing antibody by focus reduction neutralization testing (FRNT) will provide a confirmatory and, in some cases, a more specific serologic assay [52, 53, 54]. For flaviviruses, seronegatives will be tested each year by IgG ELISA [55]. The first positive IgG ELISA to DENV or ZIKV will trigger FRNT testing to determine immunity to specific viruses. For flavivirus IgG positive subjects, further assessment for interval infection will rely solely on FRNT testing. CHIKV IgG conversion will be confirmed with neutralization testing by FRNT.

Mosquito Infection: The low indoor density of *Ae. aegypti* [48, 56] would make it too costly to quantify ABV infection at the household level. Therefore, we will quantify natural ABV infection

rates in *Ae. aegypti* at the city block level (9 blocks per cluster * 50 clusters = 450 blocks monitored monthly). At the UCBE lab, we will pool all female *Ae. aegypti* collected in a block in vials containing 25 mosquitoes (if more than 25 females are collected in a city block, we will make more than one vial). Vials will contain RNALater (Quiagen) and will be stored at -80°C for future testing, which will occur at the end of each transmission season (January-June). RNA will be extracted with an QIAmp Viral RNA kit (QIAGEN, 52904). We will implement RT-PCR procedures [50] for ABV detection using a StepOne Plus (Applied Biosystems) thermocycler.

Insecticide Resistance. Standard CDC bottle bioassays [57] will assess phenotypic resistance of adult *Ae. aegypti* from treatment and control clusters pre-spraying and at 3 and 9 months post-spraying every year. We will generate 10 *Ae. aegypti* colonies using eggs collected from 10 randomly selected clusters (5 intervention and 5 control). F1 or F2 progeny from each colony will be screened for susceptibility to the following insecticides: deltamethrin, bendiocarb and pirimiphos-methyl using the diagnostic doses (DD) and diagnostic times (DT) previously established for *Ae. aegypti* by the CDC [57]. When resistance is detected, both DNA and RNA will be analyzed from a subset of the phenotyped mosquitoes to calculate the frequencies of known resistance alleles as well as expression of resistance-associated genes. This will enable us to measure changes in resistance mechanisms over time and in response to insecticide pressure. All resitance typification activities will occur at the Entomology lab at CDC in Atlanta, GA.

5.2.2 Specimen Collection, Preparation, Handling and Shipping

5.2.2.1 Instructions for Specimen Preparation, Handling, and Storage

Special instructions for the collection, labeling, preparation, handling, and storage of specimens are detailed in the Manual of Procedures attachment.

5.2.2.2 Specimen Shipment

We will be shipping serum samples from UADY to Emory for advanced and confirmatory serological assays. We estimate such shipments to occur 2x/year, coinciding with the end of the transmission season and the end of the annual sero-survey. Also, we have included *specific SOPs in the Manual of Procedures for handling blood specimens.* Mexico allows human specimen sample export to the US as long as a permit is signed by COFEPRIS (the Mexican equivalent to FDA). Approvals are protocol-specific, and we will be submitting paperwork specifically for this study.

6 STATISTICAL CONSIDERATIONS

6.1 Study Outcome Measures

Outcome measures are summarized in the following table:

Type	Name	Time Frame	Brief Description
Primary	Laboratory-confirm	Post-treatment	Laboratory-confirmed (virologically [RT-PCR testing of acute samples] or serologically [IgM and IgG ELISA testing of paired acute and convalescent samples]) symptomatic DENV, CHIKV or ZIKV.
Secondary	Laboratory confirm	Post-treatment	Laboratory confirmed (serologically, [IgG ELISA and neutralization testing of annual surveillance samples]) DENV, CHIKV or ZIKV infection
Secondary	Aedes aegypti infec	Post-treatment	Ae. aegypti mosquito infection rates with DENV, CHIKV, ZIKV (assessed by RT-PCR)
Secondary	Aedes aegypti infes	Post-treatment	Ae. aegypti indoor entomological indices (adult presence and abundance, female presence and abundance, bloodfed female and abundance)
Secondary	Community accepta	Post-treatment	Percentage of households receiving the intervention that would recommend it to others in the community (assessed by household surveys)
Secondary	Safety profile	Post-treatment	Percentage of households receiving the intervention that had evidence of a reaction to the insecticide (assessed and confirmed by study doctors)

The primary endpoint will be binary (infected yes/no) and will be used as is for the Cox proportional hazards model. Additional variables will include date of onset of symptoms as well as house and cluster ID. No transformations will be made on such variables.

For secondary endpoints, epidemiological data will also be binary (interval seroconversion, which is the combined assessment of the serological status of a participant between annual serosurveys) and used in logistic models without transformation.

For infection in mosquitoes, we will collect binary data (a mosquito pool is either infected or not), and transform it into minimum infection rates (MIR): (1/# mosquitoes in the pool)*1,000. This measure assumes that, when a pool is positive, it was due to at least one mosquito. That is why it is called ‘munimum’.

For mosquito collections, data will be collected as counts (number per house) and used as is for negative-binomial analysis or used to calculate infestation (binary at the house level, with a house being infested or not). Both measures will be used as described in [34].

We will perform household surveys to assess the acceptability of the intervention for those households receiving it. We will quantify the % of heads of household that would recommend the intervention to others.

Upon receipt of a sign of potential health reaction to insecticides used, doctors will visit the household and assess whether it could be related to the application. The % of households reporting signs of potential intoxication will be quantified for houses receiving the intervention.

6.2 Sample Size Considerations

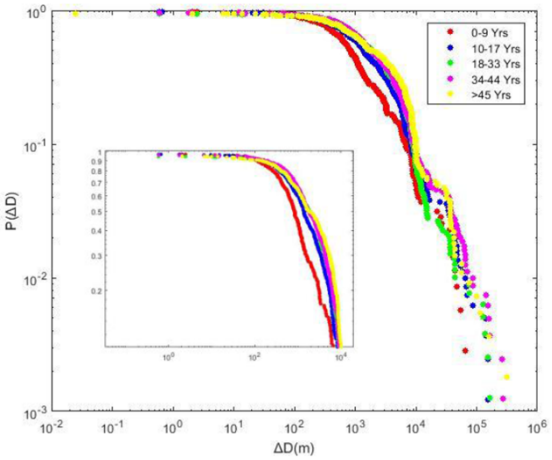


Fig. 7. Probability of moving a given distance from the home (y-axis) as a function of the distance from home (x-axis) for Merida residents, and stratified by age group (Vazquez-Prokopec et al. unpublished).

The size of clusters for the intervention will be 5x5 city blocks. This extent was chosen because: a) the area is large enough to contain more than the 92 age-eligible children required for the epidemiological evaluation; b) *Ae. aegypti* dispersal is limited, rarely exceeding 100m[58], thus concentrating our evaluation in the center of the cluster will provide a less biased estimate of intervention efficacy; c) prospective mobility surveys informing how human mobility may affect the placement of clusters. Surveys performed on 800 FSD residents showed that FSD cohort participants living in Merida had about 60% their mobility occurring within 1km of the house (Fig 7). Given the large extent of movement, separating clusters by distance (e.g., larger than 1 km separation) would be unfeasible. Instead, our approach for the trial was to focus on a “fried egg” design, where entomology and epidemiological follow-up of study participants will occur within the central 3x3 city blocks of the 5x5 city-block cluster (Fig 8).

Fig. 8. Structure of a study cluster. Yellow areas will receive TIRS, whereas blue areas will receive TIRS and participate in the epidemiological and entomological evaluation of the intervention.

<u>Randomization:</u>

The study area includes 286 hot-spot census tracts, 192 of which have total population size of at least 1,000, per the 2010 census. Census tracts are categorized into four sectors. For each census tract, we have characterized the following features that are used as balancing factors:

- Population size, per 2010 census.
- Population density, per 2010 census.
- Percent employed population, per 2010 census.
- Cumulative number of ABD cases between 2008 and 2016 (per passive surveillance).

We simulated 1,000,000+ random allocation patterns (25 clusters in group A, 25 clusters in group B – allocation to TIRS/routine vector control determined later), and we use covariate-constrained randomization[41] to eliminate the allocation patterns with an imbalance of ±10% on any of the continuous balancing factors listed above. Specifically, for each balancing factor, if for a given allocation pattern, the mean of group A divided by the mean of group B was greater

than 1.1 or less than 1/1.1, that pattern would not be considered for potential selection. Furthermore, we eliminate any allocation pattern with imbalance in the number of clusters per arm per sector greater than ±1. We will implement checks to satisfy the criteria that the design is not overly constrained, as proposed by Moulton[41] (e.g. pairs of clusters always or never appearing in the same arm). For each retained allocation patterns, we calculate the mean distance from the centroid of each cluster to the centroid of its nearest neighbor also in the trial. We restrict to the top 10% of allocation patterns with the largest mean distance. Preliminary exercises returned over 1,500 allocation patterns satisfying all selection criteria. Sample allocation patterns are plotted in Fig 9.

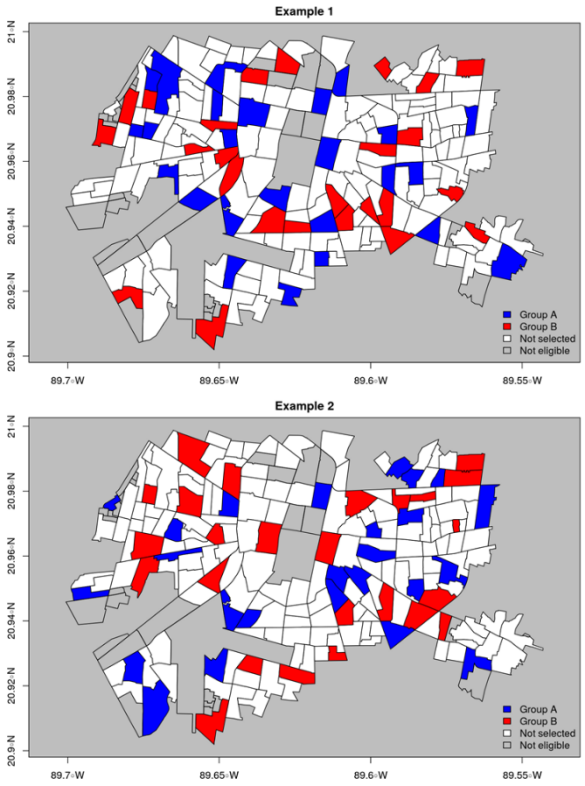


Fig 9. Two examples of outputs from the constrained randomization into two groups.

Given the set of allocation patterns that meet the above balancing criteria, we will use equal probability sampling to randomly select one allocation. For participant enrollment, the study teams will be provided with a list of 50 census tracts for inclusion in the study, without record of which census tracts are in Group A or B. At this time, households and individuals will be invited to participate in the study. No participant or investigator will have knowledge of their allocation. **After completing the enrollment process, we will then use a random number generator to determine which group (A or B) is allocated to TIRS, where each group has a 50% probability.**

Power and sample size

To assess power and sample size requirements, we analyzed historical passive surveillance data from the 192 hot-spot census tracts with population size of at least 1,000 (from our publication characterizing the ABV hot-spot area [40]). We use yearly data from 2008 to 2016 on the number of dengue, chikungunya, and Zika cases recorded in children 0-14 years each year by census tract[40]. Data were combined into pairs of adjacent years to mimic a two-year trial period. The table below summarizes the mean incidence (number of cases over two-year period/number of children) and intracluster correlation coefficient (ICC) for a given two-year period.

Data source	Mean	ICC
2008+2009 Dengue	0.0402	0.0345
2009+2010 Dengue	0.0530	0.0289
2010+2011 Dengue	0.0572	0.0164
2011+2012 Dengue	0.0847	0.0153
2012+2013 Dengue	0.0729	0.0188

2013+2014 Dengue	0.0455	0.0256
2014+2015 Dengue/Chik	0.0581	0.0229
2015+2016 Any	0.0385	0.0151

For our sample size calculations, we conservatively assume a low incidence (4% over a two-year period) and high ICC (0.035). Sample size is shown below for 50 clusters, considering 80% power and 90% power and TIRS efficacy from 70 to 90%. Sample sizes are adjusted for the study design effect and expected 20% loss to follow-up (LTFU).

POWER	TIRS EFFICACY	NUMBER OF EVENTS	TOTAL EFFECTIVE SAMPLE SIZE	NUMBER PER CLUSTER, UNADJUSTED	NUMBER PER CLUSTER, ADJUSTED FOR LTFU	TOTAL SAMPLE SIZE, ADJUSTED FOR LTFU
90%	70%	38	1390	>500		
	75%	30	1164	122	152	7600
	80%	24	984	62	77	3850
	90%	16	714	28	35	1750
80%	70%	28	1038	74	92	4600
	75%	22	870	43	54	2700
	80%	18	734	30	37	1850
	90%	12	534	17	21	1050

Assuming 4% incidence over a two-year period, 70% TIRS efficacy, an ICC of 0.035, and 20% loss to follow-up, we will require 92 age-eligible children enrolled per cluster for an overall sample size of 50 clusters and 4,600 children to have 80% power to detect a significant reduction in ABV incidence with TIRS.

Figure 10 below summarizes the distribution of the number of children less than or equal to 14 years of age in each cluster, based on the 2010 census. The minimum size is 145 children. The median is 546 children.

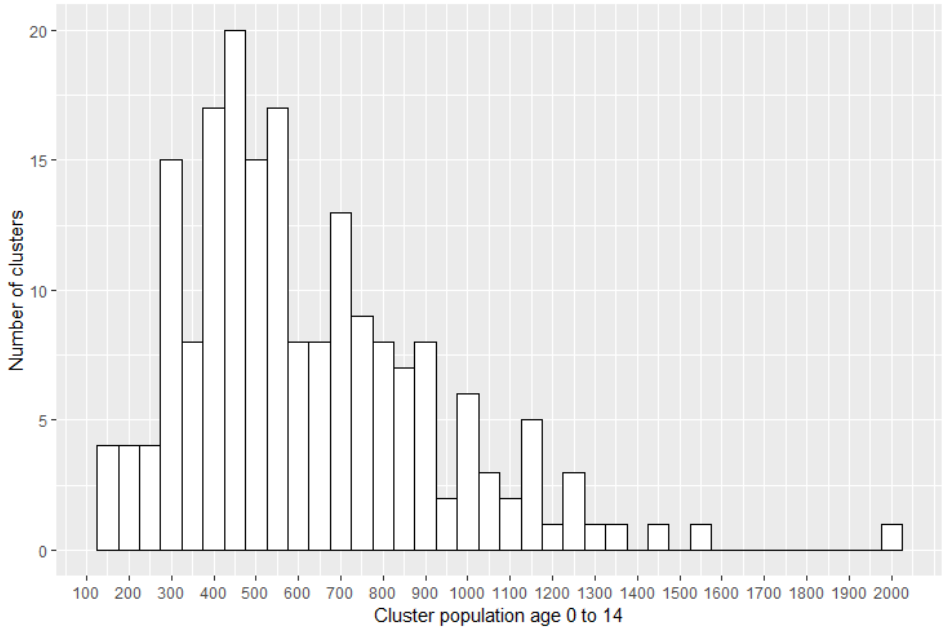


Figure 10. Distribution of children aged 0-14 per cluster, based on 2010 census.

6.3 Participant Enrollment and Follow-Up

The duration of individual participation in the trial will vary, but will not be longer than 4.5 years. Enrollment will occur at the end of Year 1 of the project, and will involve a baseline serological characterization of the population. Since then, and for the following 2-3 years, participants will be involved in the epidemiological assessment of TIRS (which will rely on active surveillance and annual blood draws). On the first two quarters of the 4th year post-enrollment, participants will provide the last annual blood sample to assess their serological evidence of interval infection. We will emphasize participants the fact that we would like people to participate as long as possible within this timeline. Febrile participants are followed clinically during the duration of their illness -- which rarely exceeds 7 days-- and we solicit convalescent samples 14-30 days after an acute sample. Movement and loss to follow-up interviews will last a maximum of 1 hour.

	2020 (Y1)				2021 (Y2)				2022 (Y3)				2023 (Y4)				2024 (Y5)			
Activity by Quarter	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Participant Enrollment																				
Baseline Serology and Entomology																				
Active Surveillance															possible 3season					
Annual sero-survey																				

6.4 Analysis Plan

Primary analysis

The primary analysis will estimate the overall efficacy of TIRS in reducing the rate of laboratory-confirmed symptomatic *Aedes*-borne disease, where the overall efficacy is estimated as one minus the hazard ratio from a Cox proportional hazards model. The Cox proportional hazards model will be fit using individual-level data for eligible and consenting children. The primary endpoint will be time to symptom onset of first laboratory-confirmed ABD. This assumes that participants having 2+ disease episodes during the trial period is rare and can be ignored. To account for clustering, the model will include a robust variance estimator with two parameters; one characterizes the level of correlation in outcomes between children within the same household, and one characterizes the level of correlation in outcomes between children in different households but within the same cluster.

The primary analysis will be the estimated overall TIRS efficacy against laboratory-confirmed *Aedes*-borne illness ($\widehat{TIRS}$). The hypothesis test for the primary outcome will be H_0: $TIRS = 0$ versus H_a: $TIRS \neq 0$. The two-sided score test will be conducted at the $\alpha = 0.05$ level. In addition, a 95% confidence interval will be calculated for $\widehat{TIRS}$.

The time origin is July 1 prior to the first season. Spraying will be completed prior to the start of the first season. The first season will extend for six months, ending on December 31. This is henceforth referred to as the *peak season*. The second season will start on July 1 of the following year and extend for six months. The at-risk process for the primary efficacy survival model will consider only time during the peak season, which corresponds to time when the residual effect of the insecticides used in TIRS is expected to be active.

We will use Schoenfeld residuals to assess departures from proportionality, as would occur if the effect of TIRS varies over time. We will use time-dependent (piecewise) models where significant non-proportionality occurs.

Secondary analyses

Epidemiological endpoints

We based our sample size calculations on the primary endpoint. Given the larger number of sub-clinical and undetected ABV infections compared to symptomatic manifestations, the study will be equally powered to detect a statistical difference in interval-level infections (measured by annual serosurveys)

Planned secondary analyses of clinical and human serological data include:

- Cox proportional hazards model with time to first laboratory-confirmed symptomatic ABV disease as the endpoint, adjusting for additional cluster- and household-level covariates (e.g. population density, household size, socio-economic status).
- Cox proportional hazards model with time to first laboratory-confirmed symptomatic ABV disease as the endpoint, adjusting for routine human movement as measured by the prospective movement survey (measured in all enrolled participants). The proportion of time in treated areas will be included as a further covariate.
- Disease-specific versions of the primary analysis (e.g. time to first laboratory-confirmed symptomatic dengue disease as the endpoint), if data permit.
- Analysis of recent human movements measured by RMS in enrolled participants presenting with symptoms for laboratory confirmation. The data will be analyzed using a test negative design-type structure, where individuals testing negative for any ABV will

serve as a comparator group for individuals testing positive for ABV. The analysis will adopt recently developed methods for cluster randomized vector control trials [81].

- Binomial generalized linear mixed effects model to assess the efficacy of TIRS for reducing laboratory-confirmed DENV, CHIKV, or ZIKV infection, analyzed as cumulative incidence over the two (or potentially three) transmission seasons, as measured from annual serological samples.

***Entomological endpoints**:*
Planned secondary analyses of mosquito data include:

- *Ae. aegypti* indoor entomological indices (adult presence and abundance, female presence and abundance, blood fed female presence and abundance) and ABV minimum infection rates (# infected mosquitoes/total mosquitoes tested x 1,000).
- Negative binomial GLMMs will test for differences in treatment and control arms for all entomologic indices as in our previous work, including *Ae. aegypti* infection rates with DENV, CHIKV, or ZIKV, and *Ae. aegypti* indoor entomological indices (adult presence and abundance, female presence and abundance, blood fed female presence and abundance) [34].
- The following *Ae. aegypti* adult indices will be calculated for each sampling date and compared between treatments and over time: presence (binomial variable) and abundance (count variable) of adults, females and bloodfed females per house. Generalized linear mixed effects models (GLMM) nested at the cluster (level 1) and city-block (level 2) levels will be used to compare each entomological index between treatment and control arms. Link functions for GLMMs will be binomial for presence indices and negative Poisson for abundance indices. The best fit models (after comparing AIC values for models including all levels or only Level 1) will be used to calculate odds ratios (OR, for mosquito presence/absence) and incidence rate ratios (IRR, for mosquito abundance) using control houses as the unit of comparison. We will calculate the operational efficacy of the intervention as E=(1-IRR)×100. This measure, ranging between 0 and 1, describes the percent reduction of mosquito abundance in treated houses with respect to the control. All models will be run with the software package lme4 within the software platform R (https://www.r-project.org/).

Transmission modeling
Our existing mathematical model for Yucatan[35, 59] will be used to simulate the effectiveness of TIRS for different scenarios of intervention coverage and insecticide residual power, using the observed trial data as a critical model input. This agent-based model of individual people and mosquitoes incorporates household demography, a spatially heterogeneous population structure based on census and remote sensing data, movement of workers and students, and seasonal fluctuations in mosquito population and incubation period.

We will model TIRS in treated households as (1) reducing the location's susceptible mosquito population and (2) increasing daily mortality probability for infected mosquitoes. For computational efficiency, we represent susceptible mosquitoes probabilistically and infected mosquitoes explicitly. For (1) we use the TIRS efficacy to reduce the susceptible population, and for (2) we calculate a daily additional mortality probability associated with the overall population reduction (e.g, roughly 0.13 for 80% efficacy TIRS). This additional mortality is treated separately from the normal daily mortality due to age or other factors. To calculate daily mortality, we use the assumed TIRS efficacy combined with our empirically-derived model for

mosquito age distribution. The age distribution determines baseline daily mortalities, to which we add a constant daily death probability.

We will use our model to evaluate the effectiveness of TIRS for different scenarios of intervention coverage and insecticide residual power. Different movement (e.g. mosquito vs. human) and transmission (e.g. pathogen introduction and elimination) dynamics become relevant at different spatial scales [16], thus we will predict the impact of scaling-up TIRS to the entire state rather than treating single cities. Simulating epidemiological trends of scaled-up TIRS for periods longer than the duration of our trial (e.g. a decade) will evaluate the effect of changing population-level immunity [35] and generate measures of effectiveness that are more informative for programmatic decision making.

Integrating ABV transmission modeling with the field study will facilitate both interpreting trial results [60] and increasing realism of the model. The explicit spatial structure of our model will allow us to separately calculate direct effects (due a person to living in a treated household) and indirect effects (due to adjacency to treated households) of TIRS as a function of residence position within, or distance from, a treated cluster. We will also use our model to evaluate competing hypotheses for unexpected field results that we observe.

Spatial spread of ABV is driven by both humans and mosquitoes, but because *Aedes* bites during the day when humans are most likely to be away from home, human movement patterns are likely particularly important in driving spatial transmission[16]. Our model already represents both human and mosquito movement, but the trial will produce data that will be highly valuable in evaluating and refining both. Survey data about human movements may be used directly by the model, while a probabilistic mosquito movement kernel may be inferred based on spatial autocorrelation patterns in ABV case data collected in the trial.

6.5 Trial Continuation Rules

The heterogeneous nature of ABV transmission may dictate the need for a third season to evaluate the epidemiological impact of TIRS. Decision to continue into a 3rd season will be made in conjunction with the program officer assigned to this project. We will follow an event-driven decision process.

After the second season evaluating TIRS, a project member (Natalie Dean) will quantify the number of total primary endpoints. We will pursue the following ranking in order to evaluate whether to stop or continue into a third season:
- If 90+ endpoints are detected, stop and analyze data as final.
- If 20-89 endpoints, continue into a third season.
- if <20 endpoints, examine feasibility/futility.

The choice of 90+ endpoints is based on our power calculations, and represents the target number of events expected for a power of 80% and a TIRS efficacy of 70%. The choice of <20 endpoints represents the target number of events needed for a power of 80% when TIRS efficacy is 90%.

7 SUBJECT CONFIDENTIALITY

The information from this study is not expected to be sensitive or lead to any negative ramifications such as legal or employment risks, and standard measures to protect confidentiality should be sufficient. All paper data forms will be stored in locked files or cabinets at UADY in a specified storage facility with limited access. All data will be stored on secure servers managed by Emory University (see below). All files sent to consultants, collaborators and co-investigators will have names and address removed. No household identifiers, beyond an anonymized household or participant code (see below) will be included within our maps or databases or shown on printed maps or in publications of the study area. Such code is produced by generating a random number for each city block (without replacement) and a random number for each house in the city block (without replacement). Then, the house ID is the combined cluster (2 characters) and block (2 characters) code (e.g., code 3355). Once house codes are generated, they won't change throughout the study. Participant codes will add two more characters to the house code (e.g., 3355##). The assignment of those last digits will be based on the order of enrollment into the study for each member of the house (e.g., 335502 will be the children enrolled second in that house). Outside of our database these codes will not be interpretable, rendering the data effectively unidentifiable without access to our servers. Access to the database will be primarily administered through a custom, web-based interface with restricted access privileges and encrypted data transfer (REDcap: https://it.emory.edu/catalog/data-and-reporting/redcap.html). Access will be limited to certified project personnel and certified associates, who will be provided unique login and password combinations. Database servers will be protected by multiple layers of security.

It will be made clear during the consent process that no information can be shared with anyone other than designated study personnel, the paper and computer files will be well protected, and we will ask that interviews be carried out one-on-one to prevent other family members listening in. We will take all necessary measures to ensure confidentiality. It will also be made clear to study personnel that any violation of confidentiality would be a fireable offense. Informed consent documents will include a specific statement relating to posting of clinical trial information at ClinicalTrials.gov, and also link the study to a ClinicalTrials.gov ID. We will permit access to all documents and records that may require inspection by the sponsor or its authorized representatives.

7.1 Future Use of Stored Specimens

The consent forms for participation in the epidemiologic evaluation of TIRS will have a separate section for requesting permission to store the participant's blood/serum for future use in studies either on dengue or other arboviruses, in view of the fact that: **a)** it may be necessary to revalidate the original laboratory findings at some point in the future; **b)** new and improved serological/diagnostic tests may become available whilst the project is in progress which would be beneficial to the project; and **c)** new viruses may be discovered over the course of the project for which it would be advantageous to screen the study participants retrospectively. The participants will be able to refuse consent for future use of their blood samples, without any repercussions. Should the participants agree to the future use of their specimens, their blood/serological samples will be stored indefinitely at secure laboratory facilities at Emory University and will not have any participant identifiers, beyond the participant's code.

8 INFORMED CONSENT PROCESS

Two levels of consent will be required for inclusion in the trial. **First**, household owners (adult member who owns or is the primary name on a lease of the property) will be presented with information materials about the intervention: the implementation of TIRS (and description of potential risks and benefits), and the entomological follow-up studies (see Manual of Procedures). After being given time to review the information, one **household owner will be asked for written informed consent to have their houses be part of the trial.** This means, they will consent to receive the TIRS intervention during at least two consecutive transmission seasons (should their house be part of a cluster receiving treatment), and to monthly entomological surveys (regardless of receiving or not the intervention). A study performed in Cairns, Australia, showed that TIRS coverages higher than 60% of houses were associated with a reduction in DENV incidence[93]. In our trial, city blocks with less than 60% consent to participate in the study will be replaced by neighboring blocks (we expect this to be rare, as our experience spraying more than 1,000 houses in Merida shows that 100% of blocks had <75% acceptance; Vazquez-Prokopec et al. unpublished).

Second, individuals from houses consenting to participate in the trial and randomly selected to be included in the epidemiological evaluation of TIRS (see section 2.4 and section 3.1.1.B) will be provided with information about the longitudinal cohort component of the trial. This evaluation will involve the prospective monitoring for symptomatic DENV, CHIKV, ZIKV illness and a yearly blood-draw for assessing DENV, CHIKV, ZIKV sero-conversion. **Parental informed consent will be obtained from children aged 2-10 and both assent to participate and a parentally signed informed consent document will be obtained for 11-15 year olds**. The consent process will be conducted by trained UADY social anthropologists already involved in the FSD cohort, who have experience working with the communities and addressing potential concerns from the population. Consent forms will be written in Spanish at an approximate sixth-grade reading level.

Consent will be obtained in participants' homes. Study personnel will visit all houses in the 5x5 city block clusters in a door to door fashion (when seeking household consent) or visit houses located in the center 3x3 city blocks of the 5x5 block cluster (when seeking individual consent). Study explanations are usually provided to small groups of adults present in the household, whereas written consent or assent are obtained from each individual participant.

Subjects may voluntarily withdraw their consent for study participation at any time without penalty or loss of benefits to which they are otherwise entitled. If a subject withdraws or is withdrawn prior to completion of the study, the reason for this decision will be recorded in a case report form (CRF). The reasons, might include, but are not limited to:

- Subject no longer meets eligibility criteria
- Subject becomes noncompliant
- Medical disease or condition, or new clinical finding(s) for which continued participation, in the opinion of the investigator might compromise the safety of the subject, interfere with the subject's successful completion of this study, or interfere with the evaluation of responses.
- Subject lost to follow-up

8.1 Informed Consent/Assent Process (in Case of a Minor or Others Unable to Consent for Themselves)

Assent to participate and a parentally signed informed consent document will be obtained for 11-15 year olds. The consent document follows Emory IRB policy for working with minors, and includes a section separating those individuals assenting from those consenting. A copy of the form is attached to this application.

9 DATA MANAGEMENT PLAN

DATA MANAGEMENT CORE (DMC):

Emory University will centralize all aspects related to data storage, management and sharing. We proposed a Data Management Core (DMC) to provide timely and efficient curation and dissemination of study data from multiple sources (e.g., clinical, laboratory, reagents, passive surveillance, entomology, demographic, Ministry of Health interventions), all essential to the success of the proposed clinical trial. The DMC will provide secure development of reproducible analytic data sets for each publication, presentation, and other research output of the study. Members of the DMC will collaborate closely with project leads and the data teams in Mexico (field and laboratory) and at Emory (laboratory) to ensure timely, accurate, and reproducible data collection, management, and analysis.

Data Management Center Personnel

Lance Waller, Ph.D., Professor of Biostatistics and Bioinformatics, Rollins School of Public Health, Emory University will serve as DMC Director. Dr. Waller has over 27 years' experience in the development, application, and interpretation of biostatistical methods to a wide range of complex public health data sets. Dr. Waller currently directs the DMCs for two large-scale NIH projects: the NIAID Tuberculosis Research Center at Emory (with PI Rengarajan and other PREVEN-TB personnel, http://tbru.emory.edu/), and the NHLBI/Gates Household Air Pollution Intervention Network trial (http://www.hapintrial.org/). Both projects involve clinical and laboratory data in domestic and international settings, and address case report forms, data transfer protocols, enrollment reporting, data quality control protocols, and long-term storage of study data.

Additional DMC personnel include: a full-time dedicated **Data Manager** to provide development, customization, adaptation, and testing of the proposed data transfer and storage platforms as well as curating our complete data holdings, project-specific research analytic data sets, and data archiving to repositories and future use. The database manager will travel regularly to Merida to coordinate all aspects of data collection and upload into the database, train database managers at UADY in proper data handling, and help with any specific data needs.

Data and Resource Management

The DMC will implement and oversee the overall plan for data storage, retrieval and curation associated with the clinical trial, including, but not limited to, clinical data from medical records, specimen tracking of human specimens, mosquitoes, intervention deployment and results of multiple types of laboratory analyses.

Fig. 1 illustrates the flow of data between study sites and provides a map for DMC data procurement, transfer, and curation plans. The DMC will collaborate with study sites and the trial leadership to refine data sharing, processing, and storage protocols for all elements of the projects, building on similar experiences with other large-scale, multi-center studies.

Secure local and cloud storage plans for our study data will be in place. Redundant Emory University-owned EMC Isilon arrays provide local storage on dedicated, secure, and HIPPA-compliant

servers. Current policies provide nightly duplication of all data from the on-campus storage array to another secure off-site storage array in downtown Atlanta. An Emory Broad Area Agreement with Amazon Web Services provides HIPPA-compliant cloud storage via a Virtual Private Network for duplication and storage of study data. We will also post specific study data associated with particular publications to appropriate online data repositories (e.g, journal archives, dryad.org, etc.). Such data will be de-identified and compliant with all our IRB requirements for data sharing and following recent recommendations for clinical trial data sharing (Ohmann *et al.* Sharing and reuse of individual participant data from clinical trials: principles and recommendations. BMJ Open. 2017 7(12):e018647. PMID: 29247106).

Analysis, Integration and Visualization

Fig. 1 also illustrates how the DMC draws data from each field, clinical and laboratory site. Data will be managed through REDCap, an open-source data capture system (developed at Vanderbilt under the auspices of NIH's Clinical and Translational Science Awards), allowing clinical data flow, reporting of completeness, and secure transfer from local REDCap servers to Emory's REDCap server. Laboratory data will be linked by research project within the LabKey system, linking heterogeneous data sources for the entire project, including REDCap clinical data objects. Data exported from the database will be exported into GIS mapping platform (QuantumGIS) for rapid visualization and spatial analytics.

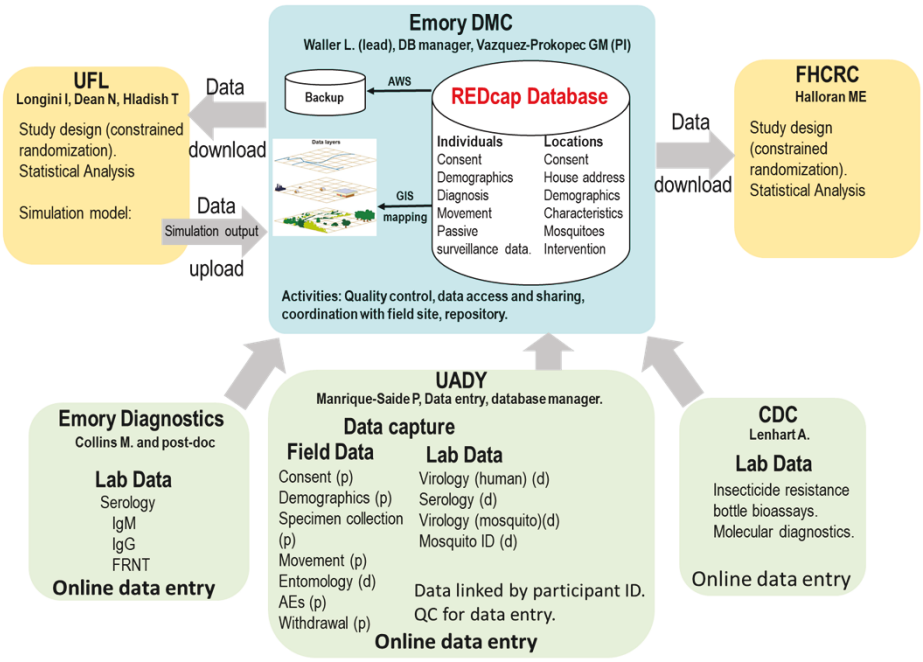


Figure 1: Structure and organization of the Emory DMC.

The DMC and lead investigators will establish data quality assurance/quality control (QA/QC) procedures, building on established QA/QC protocols for ongoing trials. The DMC will coordinate practices across existing collaborations in order to document and implement the specific QA/QC guidelines for the trial. These protocols will provide standardized data reports to monitor enrollment and progress toward study goals as well as visual analytics for each project. The DMC will provide such data reports on a regular basis to project investigators and overall study leadership.

As noted above, the DMC will also work to develop archived analytic data sets for each publication/presentation conducted using trial data. These analytic data sets provide a snapshot of all relevant data, meta-data, and code for a particular research output (e.g., manuscript) to establish reproducible research results. For example, if a researcher compares newer results to an existing publication, they need access to the data and code used in the original analysis, and any assumptions, compromises, decisions influencing the results. For primary analyses, the DMC will publish peer-reviewed "data descriptors" in data science journals (e.g., *Scientific Data*) to facilitate future replication and extension of trial results.

Transfer of Data and Reagent/Resource Information

Managing the data transfer infrastructure requires both flexibility and stability in order to respond to project needs and to maintain standards across study sites and personnel. DMC personnel are well-versed in this balance and bring an array of technical, analytical, and diplomatic skills to the project. The DMC will participate fully in discussions with the PI, leadership team and individual project leaders to establish workable and reliable transfer protocols, building on the data management infrastructure.

The DMC will ensure transmission of study data in accordance with HIPPA and other data security requirements. Where possible, the DMC will utilize secure APIs to connect analytic components, and where not possible, the DMC will establish data transfer protocols via alternative approaches (e.g., secure FTP, secure cloud storage, etc.) in order to enable, monitor, and document regular transfers so that data holdings are never more than 1-2 weeks out of date. This practice builds on past success in previous DMC projects.

Some study projects involve the transfer of specimens from clinical to laboratory sites. In addition to material transfer agreements, the DMC will work with the PI leadership team and study investigators to establish and implement specimen tracking and storage across trial-based studies.

Data and Research Resources Availability

Finally, the DMC will manage data resources locally within Emory's secure local and cloud storage servers, including the analytic data sets associated with particular research output as mentioned above. These analytic data sets provide working data for ongoing manuscript and presentation development, and also provide a record of the trial's research output. The DMC will work with investigators to provide relevant meta-data for each analytic data set and archive the relevant analytic code.

Making Data Publicly Available

Finally, the DMC will collaborate with trial investigators to upload data to proper databases for sharing with the research community in a timely manner. The DMC will develop metadata following NIH guidelines to capture protocols for the overall study and individual samples. The analytic data sets for each publication, presentation, or other research output will allow transfer of data and figures to study and NIH leadership without corruption. This metadata will link each analytic data set (to be shared with study leadership and the broader community) with all components of each trial-associated component.

10 LITERATURE REFERENCES

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SUPPLEMENTS/APPENDICES

- *Consent and assent Forms, including Future Use Consent*
- *Manual of Procedures*
- *Data Safety and Monitoring Plan (DSMP)*
- *Forms for DSMP*