Sanitation, Water, and Instruction in Face-washing for Trachoma II (SWIFT II)

MANUAL OF PROCEDURES

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The Carter Center
Amhara Public Health Institute
Emory University

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Chapter 1

Introduction

Trachoma is among the most realistic candidate diseases for elimination. Trachoma has already been eliminated in almost all industrialized countries, likely because of economic development with subsequent improvements in hygiene and sanitation. Elimination is increasingly possible in less developed settings as well, given the availability of donated azithromycin for mass distributions. Azithromycin is extremely effective at clearing the ocular strains of chlamydia that cause trachoma, and mass distributions greatly reduce the community burden of infection.

Trachoma elimination may only be possible if antibiotics are combined with water, sanitation, and hygiene (WASH) improvements. While mass azithromycin distributions are undoubtedly effective, antibiotics alone may be insufficient for elimination. The WHO recommends 3-5 annual rounds of mass azithromycin, but some hyperendemic settings have been receiving annual treatments for over a decade and still have not eliminated trachoma. Re-infection commonly occurs after azithromycin treatments in these settings, suggesting that transmission has not been interrupted. Transmission occurs from direct contact of ocular secretions, classically through "fingers, flies, and fomites." Eliminating these transmission pathways is thought to require changes to hygiene behaviors as well as improvements to the community's water and sanitation infrastructure. Yet given the long duration of chlamydial infections, it is unlikely that WASH interventions by themselves will lead to rapid elimination. The key may be to combine antibiotic distributions with WASH improvements. This is the crux of the WHO's SAFE strategy (Surgery, Antibiotics, Facial cleanliness, Environmental improvements), but few interventional studies have ever tested this hypothesis.

An ongoing randomized trial is currently testing the efficacy of WASH for trachoma in the absence of antibiotics. Aim I of the Sanitation, Water, and Instruction in Face-washing for Trachoma (SWIFT I) (NCT02754583, Keenan PI) is a cluster-randomized trial being conducted in the Amhara region of Ethiopia in which 20 clusters have been randomized to a WASH intervention and 20 clusters to no WASH intervention. Aim I of SWIFT is commonly called WASH Upgrades for Health in Amhara I (WUHA I). The intervention consists of a community water point and hygiene promotion workers, household wash stations, soap distributions, and WASH manual, and a school WASH curriculum. Clusters had received 8 rounds of annual mass azithromycin distributions before the start of the study, but no antibiotics are being distributed during the trial. Preliminary results suggest that elimination will not occur by the final

time-point (January 2019).

Our long-term goal is to eliminate trachoma even in the most hyperendemic communities. Doing so will require investigation of the best interventions for elimination as well as the optimal methods for surveillance once elimination has been achieved. With SWIFT II, we test whether the combination of antibiotics and WASH improvements will be more effective than antibiotics alone, and also perform a diagnostic accuracy study of several low-cost tests for ocular chlamydia. We propose to extend our ongoing cluster-randomized trial by continuing our WASH intervention, performing annual mass azithromycin distributions, and following communities for an additional three years. The primary outcome will be chlamydial infection with a quantitative PCR test performed in a reference laboratory, but we will also assess for trachoma with chlamydia serology from dried blood spots, low-cost conventional and quantitative PCR and LAMP tests performed in the field, and conjunctival photographs. Our central hypothesis is that combining a comprehensive WASH package with antibiotic distributions will be more likely to lead to elimination than antibiotics alone.

1.1 Specific Aims

Specific Aim 1: To determine the benefit of WASH for trachoma control, when used in conjunction with mass antibiotic distributions.

- **Hypothesis**: The prevalence of ocular chlamydia will be more likely in communities randomized to WASH plus mass azithromycin compared to communities receiving antibiotics alone.

Specific Aim 2: To compare the diagnostic accuracy of inexpensive point-of-care trachoma tests.

- **Hypothesis:** A point-of-care PCR system will be more sensitive and specific than the current standard of conjunctival grading for follicular trachoma.

This proposal presents an opportunity to leverage our existing WASH intervention and study infrastructure to assess what has long been dogma among trachoma experts: that elimination will occur only by combining antibiotics, facial cleanliness, and environmental improvements. Furthermore, we take advantage of the monitoring in the trial to compare several novel tests that could be used by trachoma programs to inexpensively yet accurately monitor the impact of their interventions. The knowledge generated should directly impact trachoma programs as they decide how to spend their limited resources to eliminate blinding trachoma.

1.2 Study Outcomes

1.2.1 Primary Outcomes

Primary outcome: The primary outcome for SWIFT II is the same as SWIFT I: ocular chlamydia as assessed by PCR, measured in a population-based sample of 0-5 year-old children at each study visit. In this study, 0-5 year old children can be taken to mean 1 day-old children to 5 year-old children. We chose the pre-school age group because previous studies have found the highest prevalence of both clinically active trachoma and ocular chlamydia, and the highest chlamydial loads, in children 5 years and under.[1–4] We use the exact same procedures that we have used in three previous clinical trials in Ethiopia; we are experienced with these methods and also in training local staff to perform the methods. Note that we had originally designated both ocular chlamydia and the clinical signs of trachoma as co-primary outcomes of SWIFT I since they are both crucial when considering trachoma elimination. However, the trial's DSMC recommended selecting the infection outcome as a sole primary outcome since ocular chlamydia is the underlying cause of trachoma, and the WASH interventions are intended specifically to interrupt the transmission of chlamydial infection. We follow their recommendation for SWIFT II as well.

1.2.2 Secondary Outcomes

The secondary outcomes of most interest are the clinical signs of trachoma on photography and the infectious load of chlamydia among those infected with ocular chlamydia. Clinical trachoma is an essential outcome because conjunctival inflammation is the ultimate cause of scarring and blindness. Moreover, clinical trachoma and ocular chlamydia infection complement each other in terms of their population dynamics: clinical trachoma provides a relatively stable, slow-changing metric of trachoma, whereas ocular chlamydia responds more quickly to interventions but is also more subject to random variation. Chlamydial load is important because Individuals with higher loads are thought to be much more likely to transmit infection.[5] We will collect data on several other secondary outcomes in children aged 0-5 years, including nasopharyngeal pneumococcal macrolide resistance, anthropometry, and the presence of a clean face on photography. We will perform similar assessments of ocular chlamydia and clinical trachoma in a sample of children aged 6-9 years and a sample of individuals aged 10 years, and similar assessments of facial cleanliness in the 6-9 year-old age group. We are monitoring for macrolide resistance in nasopharyngeal pneumococcus because azithromycin treatments may select for antibiotic resistance and because of the possibility that improved hygiene will have an added benefit of reducing the carriage of nasopharyngeal pneumococcus. We are monitoring anthropometry because of the possibility that improved hygiene will result in accelerated growth in pre-school children. Our group has measured both nasopharyngeal macrolide resistance and anthropometry in previous trials in Ethiopia.[6–8] Another important secondary outcome is improvement in conjunctival inflammation as assessed from serial conjunctival photographs, measured in a longitudinal cohort of children aged 0-5 years at baseline.

1.3 Research Plan

We are extending our ongoing cluster-randomized trial (SWIFT I) by performing a mass azithromycin distribution after the final study visit and following communities for an additional four years. Forty communities that had received 8 rounds of mass azithromycin distributions were randomized in a 1:1 ratio to the WASH arm or a control arm in WUHA I (randomization performed January 2016). The WASH package includes a community water point, community-based hygiene promotion workers, household wash stations, household WASH education books, household soap distribution, and a hygiene curriculum for primary schools. Annual monitoring visits are conducted in each community, with the final WUHA I 3-year study visit taking place in February 2019. For WUHA II, the primary outcome is ocular chlamydia infection among 0-5 year-old children at months 48, 60, 72, and 84. A secondary outcome is the load of ocular chlamydia among infected children, assessed at the same time points. It is worth noting that the WUHA II design is not dependent on the results of WUHA I. If the WASH intervention is found to be effective in WUHA I, then WUHA II will determine whether trachoma elimination is possible when combining WASH with mass antibiotics. If we fail to find a significant benefit of WASH in WUHA I, then WUHA II will provide longer follow-up to allow the behavior changes in the WASH arm to take effect.

We will continue the intervention in the 20 clusters of households that have already received the intervention as part of SWIFT I. We will continue to monitor chlamydial infection among the 20 WASH intervention and 20 delayed WASH intervention clusters from the SWIFT I trial. The primary outcome will still be chlamydial infection with a quantitative PCR test performed in a laboratory, but we will also assess for trachoma with chlamydia serology from dried blood spots, low-cost conventional and quantitative PCR and LAMP tests performed in the field, and conjunctival photographs. Our central hypothesis is that combining a comprehensive WASH package with antibiotic distributions will be more likely to lead to elimination than antibiotics alone. We also hypothesize that the diagnostic accuracy of inexpensive point-of-care trachoma tests will be more sensitive and specific than the current standard of conjunctival grading for follicular trachoma.

Chapter 2

Background and Rationale

Trachoma is an ancient disease that remains the most common infectious cause of blindness worldwide.[9] Over 40 million people are currently affected by active trachoma, with the highest burden in sub-Saharan Africa.[10] Trachoma is caused by ocular infection with Chlamydia trachomatis: conjunctival chlamydia infection causes inflammation, with characteristic white follicles (germinal centers) on the upper tarsal conjunctiva. Frequent bouts of infection and inflammation result in conjunctival scarring, entropion (in-turning of the eyelids), and trichiasis (eyelashes touching the globe). Trichiasis is painful, and leads to corneal abrasions, bacterial and fungal superinfections, corneal opacity, and blindness. The National Eye Institute's most recent 5-year plan encourages clinical trial interventions for blinding global eye diseases, and specifically urges research in connection with trachoma control programs to cost-effectively test optimal intervention strategies for global infectious causes of blindness.[11]

The World Health Organization (WHO) has called for elimination of trachoma as a public health problem by the year 2020.[12] Through its Global Elimination of Trachoma by 2020 (GET 2020) initiative, the WHO has designated trachoma a priority eye disease, and has brought together academic institutions, non-governmental organizations, and pharmaceutical companies to promote its elimination. Together, this consortium supports the SAFE strategy: Surgical correction of trichiasis, Antibiotics to reduce the community load of ocular chlamydia, and Facial cleanliness and Environmental improvements to reduce the transmission of ocular chlamydia.[13] Most trachoma experts are enthusiastic that trachoma can be eliminated in the next two decades, and some even think that trachoma may be a candidate for global eradication.[14]

Mass azithromycin distributions are clearly effective in reducing the burden of ocular chlamydia, but have not proven to be the magic bullet for elimination. Numerous community-randomized trials performed in a variety of settings have found dramatic reductions in ocular chlamydia following mass treatments, supporting the WHO's recommendation of 3-5 annual rounds of treatment in areas with endemic trachoma.[1,15–18] Mass azithromycin distributions may be capable of eliminating ocular chlamydia infections in areas with hypo- or meso-endemic disease, but have not resulted in long-lasting elimination areas with hyperendemic trachoma. Some areas of Ethiopia have been treated annually for over a decade and have not experienced elimination. More frequent treatments should speed elimination according to mathematical models, but even 8 years of biannual treatments were not sufficient to eliminate chlamydial infection

in our previous study in Ethiopia. It is becoming clear that antibiotics alone will not eliminate trachoma in the most severely affected regions.

Few randomized trials have assessed the impact of WASH improvements on trachoma, and those that have been performed have not been able to demonstrate a reduction in ocular chlamydia. Although this may mean that water and sanitation interventions have no effect on ocular chlamydia, it is also possible that the limitations of these trials prevented a true effect from being found. For example, the trials were typically underpowered and had short follow-up. None provided all three WASH components (e.g., water, sanitation, and hygiene) in concert.[19,20] Most used clinically active trachoma as the outcome, based on a field grading scale that may not be a sensitive enough test.[21,22] The state of evidence regarding environmental modifications for trachoma control is perhaps best summarized by the 2012 Cochrane Review article on this subject, which concluded: "Generally there is a dearth of data to determine the effectiveness of all aspects of environmental sanitation in the control of trachoma."[23] Perhaps because of this, many trachoma programs focus their activities around the "S" (surgery) and "A" components (antibiotics), while neglecting the "F" (facial hygiene) and "E" (environmental improvements) components of the SAFE strategy.[24]

Elimination of trachoma will require not only effective interventions, but also accurate surveillance. Trachoma programs currently rely on district surveys in which trained field workers assess for follicular trachoma (TF) in 1-9 year-old children. Decisions on initiating and discontinuing interventions are based on the prevalence of TF during these surveys. However, the prevalence of TF in a community does not correlate well with the prevalence of ocular chlamydia, and this correlation is even worse if mass azithromycin treatments have recently been administered. The discrepancy is thought to result from differential kinetics of infection vs. inflammation: infection clears quickly after antibiotics, but inflammation takes much longer. Direct tests of ocular chlamydia may be preferable, since interventions are directed toward reducing transmission of ocular chlamydia. Nucleic acid amplification tests (NAATs) have generally been too expensive and complicated for field use, but inexpensive and portable point-of-care NAATs are increasingly becoming available, which could revolutionize diagnostics in resource-limited settings.

The SWIFT I trial, a three-year, NEI-funded, cluster-randomized trial aims to determine the impact of WASH on ocular chlamydial infection. Our intervention consists of several community-level interventions, including a community water point and a staff of hygiene promotion workers who travel door-to-door to educate community members. Each household received a wash station (jerry can with faucet plus a mirror) and a 66-page educational WASH book, and each household receives a monthly supply of soap. We also implemented a hygiene curriculum for the primary schools in the study clusters, complete with educational aids. We monitor the fidelity of our intervention and fine-tune its components through spot-checks of households and schools, focus group discussions, structured observations of hygiene behaviors, and an annual household survey. Our monitoring has revealed a high uptake of the intervention as well as evidence of subsequent hygiene behavior changes. Given these initial successes, and given the paucity of long-term randomized trials of WASH interventions for health outcomes, we propose a continuation of the existing WUHA trial with annual monitoring for an additional three years in order to more accurately gauge the true impact of WASH on trachoma.

The contributions of the trial are twofold: first, to determine the long-term benefit of WASH for trachoma when combined with antibiotics, and second, to explore possibilities for low-cost, highly accurate point-

of-care test for chlamydia. We are extending the WUHA trial by continuing the WASH intervention. We are also performing annual mass azithromycin distributions in all study communities. We will continue to monitor for ocular chlamydia via PCR of conjunctival swabs and serologic testing from dried blood spots. We ask whether antibiotic distributions combined with a comprehensive, well-functioning WASH package is more likely to eliminate trachoma than antibiotics alone. We will also collect one additional swab on each individual during routine monitoring visits and compare several inexpensive, commercially available nucleic acid tests for chlamydia. The proposed trial leverages our existing research infrastructure and takes advantage of the fact that the intervention has already been implemented, and will have been operating for more than five years by the end of the proposed study. WASH interventions are thought to take a long time to work given their reliance on changing behavior, and thus we will increase the chances of finding an effect if one truly exists. Moreover, we will advance knowledge regarding trachoma surveillance, which has become increasingly important as the world moves towards global elimination. We think the results of the proposed study will be of great interest to the trachoma community, and regardless of the outcome will directly help trachoma programs decide how to spend their limited resources.

Chapter 3

Administrative Information

3.1 Trial Registry

The clinicaltrials.gov registry number is NCT02754583.

3.2 Funding

SWIFT I is funded by the National Eye Institute. (U10 EY023939). SWIFT II is also funded by the National Eye Institute (UG1 EY023939).

3.3 Roles and Responsibilities

3.3.1 Protocol Contributors

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3.3.2 Trial Sponsor

The NEI is sponsoring this trial. The NEI has no role in the collection, management, analysis, and interpretation of data; writing of the report; or the decision to submit the report for publication, and does not have ultimate authority on these activities. The sponsor has representation on the Data and Safety Monitoring Board.

3.3.3 Study Partners

The Proctor Foundation and Carter Center are currently collaborating on the WUHA I trial, and have worked together on 5 other large NEI- or Gates-funded cluster-randomized clinical trials since 2006 in Ethiopia and Niger. The collaboration has been very productive, with numerous publications in high impact journals. The research partners complement each other, with the Proctor Foundation providing expertise in study design and analysis, and the Carter Center implementing the field work in a thorough yet efficient manner. Dr. Matthew Freeman from Emory University is continuing to collaborate on SWIFT II. Dr. Freeman is the WASH expert for WUHA I/II, and he has conducted cluster-randomized trials of WASH interventions in Africa. He has been an invaluable member of the WUHA team. Diana Martin from the CDC will continue to process the chlamydial serology samples. Dr. Martin helped developed the technology and has collaborated with Proctor researchers in previous studies.

Francis I. Proctor Foundation

The Proctor Foundation is an organized research unit at the University of California, San Francisco. The Foundation has a 73 year history of research in ocular infectious and inflammatory diseases and runs one of the leading corneal fellowship training programs in the United States. Proctor Foundation faculty have been involved in prevention of blindness research in developing countries since the foundation's inception. The impetus for establishing the foundation in 1947 was to eradicate trachoma in the American Southwest and in other parts of the world.

From this initial vision we have expanded our research efforts to include the other major causes of blindness worldwide, with a continuing emphasis on infectious and inflammatory eye diseases. The Proctor Foundation will be the main coordinating center for the study. Dr. Jeremy Keenan, Principal Investigator of the study at Proctor Foundation, will be assisted by numerous co-investigators, a research

coordinator, a data management team, and a biostatistician.

Our experience working in Ethiopia has been very successful to date – with the TEF (Trachoma Elimination and Follow-up) study in the Gurage region, TANA (Trachoma Amelioration in Northern Amhara) and the TIRET (Tripartite International Research for the Elimination of Trachoma) study in the Amhara region. We have partnered with ORBIS International and The Carter Center, respectively, to conduct/complete these large-scale projects.

The Carter Center – Ethiopia

The Carter Center is guided by a fundamental commitment to human rights and the alleviation of human suffering; it seeks to prevent and resolve conflicts, enhance freedom and democracy and improve health. The trachoma control program does not just fight disease, it fights the conditions that perpetuate disease: poverty, poor sanitation, lack of knowledge, and hopelessness. Together, the Carter Center and its program partners are working to build a brighter future for those at risk for this devastating disease.

The focus of the country program has been on trachoma control, and delivery of quality eye care and blindness prevention programs to the majority of the population that live in rural areas (72%), largely unserved by trained eye care professionals.

Partnering with the Carter Center has yielded very positive results. The organization has an established infrastructure for large scale health promotion and improvement programs, as well as a strong working relationship with the Ethiopian Ministry of Health – both of which have contributed to the success of our field research.

Amhara Public Health Institute (formerly Bahir Dar Regional Health and Research Laboratory)– Ethiopia

The Amhara Public Health Institute in Bahir Dar, Ethiopia was established to be a center of excellence for the development of laboratory systems in the region. Construction of this CDC and USAID supported laboratory was completed in 2011. Conjunctival swabs will be processed with the Abbott RealTime assay for Chlamydia trachomatis, using the automated Abbott m2000 System, which is already in use at the laboratory. The Amhara Public Health Institute will also process the swabs using media selective for Streptococcus pneumoniae, and then test for antibiotic resistance to erythromycin, penicillin, tetracycline, and clindamycin using the Kirby Bauer disc diffusion assay.

Emory University

The Rollins School of Public Health comprises six academic departments: behavioral sciences and health education, biostatistics, environmental and occupational health, epidemiology, health policy and management, and global health. It also hosts over 20 interdisciplinary centers. Its location in Atlanta provides ample interaction with other public health professionals interested in global health. Specifically, the

school has close ties with the Carter Center and the Centers for Disease Control and Prevention, both of which are in Atlanta and contribute faculty to the school. Dr. Matthew Freeman has a appointments in the Department of Environmental Health, the Hubert Department of Global Health, and the Department of Epidemiology.

Centers for Disease Control and Prevention

Dr. Diana Martin and her lab at the Centers for Disease Control and Prevention are developing new serological tests to monitor trachoma elimination as well as other diseases of interest including helminths, intestinal bacterial pathogens, and intestinal protozoal pathogens. Dr. Martin's lab will complete serological testing for trachoma and other diseases of interest using the dried blood spots collected throughout the study.

3.3.4 Study Committees

Executive Committee

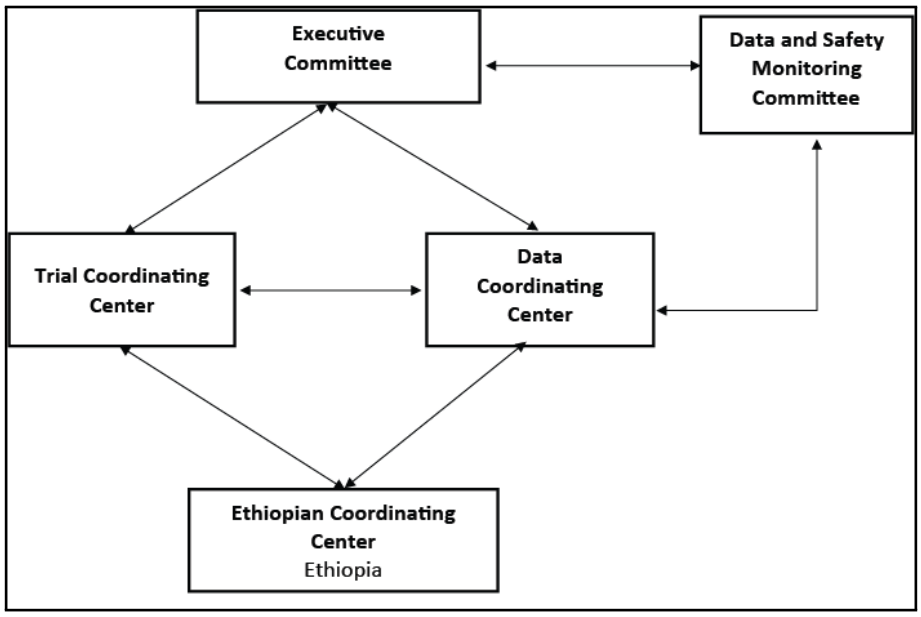
The Executive Committee will consist of Drs. Jeremy Keenan and Zerihun Tadesse. This committee will act as the administrative and executive arm of the clinical trial and will meet twice per year to provide overall oversight for the study and make decisions on day-to-day operation issues as described in the following:

- Monitor study progress and data collection process
- Discuss any quality control issues that have arisen in the Trial Coordinating Center (TCC) and Data Coordinating Center (DCC)
- Evaluate and adopt changes in study procedures as necessary
- Communicate with and implement recommendations from the Data and Safety Monitoring Committee (DSMC)
- Make executive decisions on the allocation of resources
- Establish policies on publications and authorship
- Approve and oversee ancillary studies

Trial Coordinating Center (TCC)

The TCC will be located at the Proctor Foundation. Dr. Jeremy Keenan will lead the center, which will also include Dr. Thomas Lietman (co-investigator), Dr. Thuy Doan (co-investigator), Dr. Travis Porco (biostatistician), Mr. Jason Melo (statistical analyst), and Mrs. Dionna Wittberg (Proctor research coordinator). The role of the TCC will be to oversee and coordinate the overall implementation of the trial. Specifically, this means:

Figure 1: SWIFT II organizational chart



- Maintaining an up-to-date manual of procedures
- Obtaining ethical approvals from all involved parties (UCSF, Emory, and Ethiopia)
- Conducting training and certification of all study personnel
- Ensuring proper masking of outcome assessment
- Monitoring adherence and adoption of the study intervention
- Maintaining a list of study personnel for group authorship, as well as organize and store consents to be included in the acknowledgement section for contributors not meeting criteria for group authorship

The TCC will organize site visits at least once per year before each monitoring visit to conduct training sessions with outcome assessors and monitor the quality of data collection. Either the principal investigator or research coordinator will be present at all site visits. The Proctor research coordinator, with assistance from the local Carter Center study coordinator, will organize the training sessions and the design and production of all educational messaging used in the hygiene promotion. This is a role she has filled for other trials in Ethiopia for the past 5 years. The TCC will meet officially as a group at least 4 times per year. All members of the TCC are currently working on studies of trachoma in Ethiopia and Niger. The group has close working relationships with the Carter Center staff in Ethiopia, and experience with cluster-randomized trials in resource-poor settings.

Ethiopian Coordinating Center

The Ethiopian Coordinating Center will be located at the Carter Center Ethiopia headquarters in Addis Ababa, Ethiopia. The center will be led by the study site principal investigator, Dr. Zerihun Tadesse,

and also include the Carter study coordinator, Dr. Solomon Aragie. Dr. Zerihun will oversee the study activities that take place in Ethiopia, and will manage the day-to-day activities of the Carter Center study coordinator. He will also assist with obtaining ethical approval from appropriate federal, regional, and zonal agencies. He is supervised by Kelly Callahan from the Atlanta headquarters of the Carter Center. The Carter Center study coordinator (Dr. Solomon Aragie) will work with the local health officials and TCCE staff to implement all study activities, including but not limited to:

- Obtain ethical approval from appropriate federal, regional, and zonal agencies
- Facilitate customs clearance of drugs, equipment, and supplies used for the study
- Hire local personnel to serve as census workers, examiners, antibiotic distributors, hygiene cluster coordinators, and health promotion workers (HPWs)
- Assist with the WASH intervention development including materials development, formative research, and focus group discussions
- Ensure that the WASH intervention is implemented and progresses per protocol.
- Liaise with study partners including Catholic Relief Services
- Supervise all data collection activities of the census, exam, and treatment phases of the study
- Obtain Material Transfer Agreement (MTA) for relevant specimens, for shipment to San Francisco
- Arrange proper storage and transport of specimens
- Coordinate with the Amhara Public Health Institute to ensure the laboratory has adequate supplies and processes the specimens in a timely fashion
- Coordinate data entry of paper forms

Data Coordinating Center (DCC)

The DCC will be located at the Proctor Foundation and led by Dr. Travis Porco. The Proctor Foundation has served as a DCC for several other trials, including cluster-randomized trials of trachoma in Ethiopia (TANA and TIRET continuation study, NEI U10 EY016214) and Niger (MORDOR; Gates OPP 1032340), and individual cluster-randomized trials of corneal ulcers in India (SCUT, NEI U10 EY015114; MUTT, NEI U10 EY018573). Dr. Porco, Dr. Keenan, Dionna Wittberg (research coordinator), and Jason Melo (statistical analyst) will be responsible for data management, data quality control, event adjudication, and training and certification of data entry personnel. The DCC's responsibilities include:

- Draft the trial's statistical analysis plan
- Develop and maintain protocol for enrollment monitoring

- Monitor data collection and enrollment with the help of Salesforce.com reports and activity dashboards. An enrollment report will be generated prior to baseline, and the data analyst will monitor this report on a monthly basis. Individual dashboards will be made to track the census, examinations, treatment progress. These dashboards will be emailed to the PI, research coordinator, data manager, data analyst, and Ethiopian study coordinator each morning during that study activity period. Dashboards will be designed to depict the following quality control metrics:
  - Census
    * Number of households stratified by date of census, census worker, randomization unit
    * Age and sex distribution stratified by census worker and randomization unit
    * Number of households with missing GPS coordinates stratified by census worker and randomization unit
  - Exams
    * Number of children examined, stratified by study population, type of swab/test, and randomization unit
  - Treatment
    * Treatment status of individuals stratified by date of treatment, treatment worker, randomization unit
    * Cross-tabulation of treatment assigned vs. treatment received
- Coordinate the use of the electronic data capture system, including maintenance of the software application and data backup
- Clean and manage the data
- Analyze data according to the Statistical Analysis Plan (SAP)
- Generate reports including enrollment, retention, and protocol violations once yearly and at the request of the EC or other study team members. These reports will be generated prior to the UCSF IRB Continuing Review application.
- Provide data requested for publications
- Coordinate and supervise the activities of the Data and Safety Monitoring Board
- Prepare interim and final data reports
- Keep in close contact with the Ethiopian study coordinator to help address any data related issues in Ethiopia.

Data and Safety Monitoring Committee (DSMC)

The DSMC has been formed according to NIH guidelines and is comprised of independent experts in bioethics, biostatistics, epidemiology, ophthalmology, and international health. The DSMC will meet at least once per year. Ad hoc meetings may be convened as needed. The DSMC approved the trial protocol at the first meeting (16 September 2014). At annual meetings, the DSMC will review data on effectiveness outcomes. A chief responsibility will be to decide whether ocular chlamydia infection levels become high enough (around 20% in study clusters) to necessitate treatment with mass azithromycin. They will also monitor for unanticipated events.

Staff Responsibilities

Principal Investigator (Dr. Jeremy Keenan, Proctor Foundation)

- Develop study design, specific aims and outcome measures, with help of biostatistician, research coordinator, study coordinator, and partners
- Obtain grant funding and with help of partners, develop grant budget
- Ensure that staff follow through on protocol and properly execute all areas of research
- Ensure that all ethical approval is maintained
- Oversee intervention implementation
- Write or add major contributions to all study-related publications
- Ensure proper masking procedure for staff involved in the study
- Supervise training certification for all examiners
- Lead the Executive Committee and Trial Coordinating Center
- Participate as a member of the Data Coordinating Center

Research Coordinator (Dionna Wittberg, Proctor Foundation)

- Develop and maintain study protocol
- Ensure the execution of the study per protocol
- Maintain all clearances for the study, including IRB renewals (UCSF, Emory, APHI, NRERC, and the Ethiopian FDA), the biological use authorization (BUA), Materials Transfer Agreement (MTA), and DSMC-related approvals
- Coordinate the activities of the Data and Safety Monitoring Committee
- Manage budgetary items including the NIH award and subcontracts
- Participate as a member of the DCC
- Assist with data collection and management
- Maintain a list of all study personnel for inclusion in study manuscripts
- Coordinate with The Carter Center, Ethiopia (TCCE) and particularly with the TCCE study coordinator, in execution of the study
- Manage correspondence between all collaborating organizations and parties
- Maintain communication and partnership with Principal Investigators regarding all study activities and plans
- Direct development and implementation of WASH intervention
- In collaboration with Ethiopian study coordinator, prepare the census and examination application, electronic tools, and paper forms necessary for fieldwork (cluster registration lists, spot check REDCap, costing forms, mini-spot check household lists, and etc.)
- Direct team, with help of TCCE study coordinator, while in Ethiopia

- With help of TCCE study coordinator, train census, exam and treatment workers on study activities including the use of the electronic data capture system
- Train and certify hygiene cluster coordinators and health promotion workers (HPWs) with help of TCCE study coordinator
- Arrange logistics and itineraries for traveling team members
- Purchase, maintain, organize, and transport of all necessary study supplies

Study Coordinator (The Carter Center, Ethiopia)

- Prior to the start of the study, secure necessary ethical and administrative approvals the Region (APHI), NRERC (Ministry of Science and Technology), and the Ethiopian FDA
- At baseline and throughout the study, obtain and maintain permission from zonal health leaders for the SWIFT study. Meet with and obtain permission/consent from all kebele and sub-kebele leaders.
- Train and supervise census and household survey team as well as the treatment team, including training in the electronic data capture system
- Supervise and train exam data collection teams (with emphasis on any new members) throughout each collection, especially after departure of Proctor study team
- Manage the data collected during the census, exams, and treatment by ensuring that the app data is uploaded to Salesforce.com and that the exam photographs are saved on external hard drives
- Arrange printing of study materials including educational materials for households, hygiene workshops, schools, and advertising
- Train, certify, and work closely with the hygiene cluster coordinators and the HPWs (with help of Proctor research coordinator)
- Maintain all fieldwork documents and records
- Complete all collection and intervention reports
- Work with Data Analyst to maintain census and exam database
- Help develop and implement WASH interventions
- Oversee transport of study samples to Amhara Public Health Institute
- Assist Proctor Coordinators in coordinating logistics in Ethiopia
- Train and supervise data entry staff at Carter Center Headquarters in Addis Ababa
- Analyze and provide data when requested by co-investigators or staff from Proctor Foundation
- Appropriately back-up all data

Co-Investigators (US and Ethiopia)

- Assume responsibility for the study in the absence of the Principal Investigator, if necessary
- Help supervise local workers, lab workers, nurses, and local health agents in the field to ensure conformity to study procedures

- Communicate with the research coordinator and principal investigator to ensure the execution of the study as per the protocol
- Fulfill responsibilities of membership on Committees (EC, TCC, and DCC) and Centers as outlined above
- Fulfill specific responsibilities as outlined in budget justifications

WASH Expert (Emory University)

- Serve as co-investigator and WASH technical advisor
- Provide technical assistance in fundamental behavioral interventions and qualitative methods
- Help design the formative research component of the study, including in-field training and analysis
- Help design the structured observations methodology, including in-field training and analysis

Biostatistician (Proctor Foundation)

- With the Principal Investigator, create the Statistical Analysis Plan (SAP)
- Receive all study data and review for quality control purposes
- Ensure appropriate masking
- Prepare data analysis plan for annual DSMC meetings. Help analyze and prepare all presented data for DSMC meetings, DSMC reports, and all SWIFT study publications

Statistical Analyst (Proctor Foundation)

- Maintain database for all collection and results-related data for the study
- Monitor receipt of all census, treatment, and exam data after each collection activity
- Develop database for entry of costing data at the Carter Center, Addis Ababa
- Track and analyze process indicators including household WASH survey, spot check data, and structured observations
- Develop consistency checks in the data management—verify any inconsistencies or questions regarding data through communication with Study Coordinator
- Analyze and provide data regarding collection and results for study staff when needed, such as for publications or DSMC meetings
- Back-up all data appropriately
- Follow-up on any missing data or lab results

Microbiologist (UCSF)

- Train laboratory technicians, especially the microbiologists at the Amhara Public Health Institute, for all lab procedures concerning the study: including Polymerase Chain Reaction (PCR) procedures, macrolide resistance testing, standard laboratory procedures for all tests, record-keeping, and quality control measures as per approved standards

- Verify equipment quality

Microbiologist/laboratory technician (Amhara Public Health Institute)

- Provide training review and supervise laboratory technicians for all lab procedures concerning the study, including PCR procedures
- Perform all macrolide resistance testing
- Follow standard laboratory procedures for all tests
- Keep records of all tests performed
- Maintain quality control measures as per approved standards
- Make sure all equipment is calibrated and maintained
- Maintain stock of laboratory reagents and supplies
- Pool and prepare all PCR samples, and process using Abbott RealTime assay for Chlamydia trachomatis, using the automated Abbott m2000 System
- Perform regular maintenance checks of the Abbott m2000 system, under direction of the UCSF microbiologist
- Report PCR results to DCC
- Maintain cleanliness and maintenance of molecular biology laboratory and all equipment

Laboratory Assistants (Amhara Public Health Institute)

- Organize all study samples for PCR processing in at APHI
- Monitor correct receipt of study samples (using corresponding exam sheets) after each monitoring visit
- Prepare samples for PCR processing
- Organize work flow of PCR processing for laboratory technicians, in consultation with program manager and principal investigator
- Maintain all samples in storage freezers; create and maintain clear map of study samples for each freezer
- Process NP samples

Hygiene Cluster Coordinators (WagHimra, Amhara, Ethiopia)

- Complete intensive on-site training from the Proctor Foundation and Carter Center Ethiopia
- Work closely with the study coordinator (TCCE) to ensure proper implementation of WASH components
- Responsible for overseeing 20 WASH clusters
- Responsible for overseeing 20 Hygiene Promotion Workers (HPWs)

- Conduct trainings for health extension workers, priests, and Health Development Army members
- Conduct monthly visits to all 20 WASH clusters
- Help with identification of potential water point locations and management of the water point construction (Water)
- Troubleshoot maintenance issues with Woreda Water Office or artisans (Water)
- Perform bi-monthly spot checks in 20 WASH clusters to check on water point functionality, school curriculum implementation, and household WASH behaviors and infrastructure (visit 8 randomly selected households with pre-school children during each visit)
- Encourage households to build a latrine and keep it in good working order (Sanitation)
- Lead latrine promotion campaigns (Sanitation)
- Training of Teachers: help conduct a training for teachers of primary schools in the study area; "train-the-trainers" model where teachers then implement activities for students (Hygiene)
- Key messages: Help conduct focus groups in clusters to identify the educational messages that will most motivate behavior change; work closely with study coordinator (TCCE) to help identify key hygiene promotion messages in hygiene promotion activities and appropriate forums for education (Hygiene)
- Monthly workshops: Supervise HPWs as they conduct monthly workshops in each cluster, with educational messages in appropriate local venues (Hygiene)

Health Promotion Workers (WagHimra, Amhara, Ethiopia)

- Complete intensive on-site training workshop from the Proctor Foundation and Carter Center Ethiopia
- Work closely with the hygiene cluster coordinator (TCCE) to ensure proper implementation of WASH components
- Responsible for WASH education in 1 cluster
- Help troubleshoot maintenance issues (Water)
- Report issues with water points (Water)
- Liaise with Water Committee (Water)
- Encourage increased water use for hygiene activities and use of safe water sources (Water)
- Perform spot checks of latrine quality during household visits (Sanitation)
- Encourage and each households how to build a latrine and keep their latrine in good working order (Sanitation)
- House to house visits: visits each household at least once per month to encourage positive WASH behavior change and improvements to hygiene infrastructure at the household level (Hygiene)
- Monthly workshops: conduct educational hygiene focused workshops in each cluster (Hygiene)
- School curriculum: work with school principal and WASH club leader to promote WASH in the schools and ensure that the schools are implementing the curriculum (Hygiene)

Health Development Army

- Local community members selected by woredas in Amhara, Ethiopia
- Assist with implementation of hygiene activities
- Participate in annual WASH promotion training given by hygiene cluster coordinators and/or HPWs
- Implement WASH promotion in 5 nearby households

Census Team

- The Carter Center will organize and train local workers to perform the census.
- During census phase, travel to enrolled clusters and obtain participant information. The census will be performed with an electronic data capture system, with the following data inputs for each household / household member:
  - Name
  - Gender
  - Age
  - School(s) any children attend
  - Water point(s) household utilizes
  - Presence and status of each household member
  - Health provider(s) household utilizes
  - GPS coordinates for each household
  - GPS coordinates for each water source in the study area
  - Presence or absence of usable household latrine and latrine-use
  - Presence or absence of washing station and soap
  - Information on hygiene behaviors (e.g., face-washing and latrine use)

Treatment Team

- Complete training by TCCE study coordinator and officials from Local Zonal Health Desk regarding proper treatment protocols and procedures
- Travel to enrolled clusters and administer study medication to the study subject per protocol
  - Directly observe consumption of azithromycin
  - Record antibiotic coverage against the previous census
  - Return to households until they have distributed antibiotics to at least 80% of selected individuals
- Inform patients of available health care facilities and procedures in local health centers and hospitals
- Collect information on the nature of any Adverse Events experienced by study subjects, and report this back immediately to investigators

Examination Team: Local workers, nurses, and lab technicians

- At each collection phase, complete training and certification in clinical examination, sample collection procedures, and data collection procedures
- Prepare all study-related materials before travel to study sites
- In each cluster, mobilize and identify all randomly selected participants
- Explain study purpose and procedure and obtain verbal consent for enrollment from each participant or participant's guardian
- Perform clinical exam for each patient and collect all participant study samples according to protocol.
- Store and record all samples correctly for transport, organization, and processing
- Counsel and motivate patients for follow-up monitoring visits
- Inform patients of available health care facilities and procedures in local health centers and hospitals
- Collect information on the nature of an Adverse Events experienced by study subjects, and immediately report to investigators

3.3.5 Protocol Revisions

Any changes to the protocol made during the course of the study will be incorporated into this Manual of Operations and Procedures (MOP), and recorded in a change log at the end of this document. Any new forms will be incorporated in addendum. The protocol changes should be submitted and approved by all relevant ethical review boards (UCSF, Emory, APHI, MoST, and E-FDA) and the DSMC.

3.3.6 Presentations and Publications

All presentations and publications should include acknowledgement of the funding sources and give credit to the collaborating organizations and/or individuals involved. Acknowledgements will include grant source(s) and the DSMC members.

Chapter 4

Research Design

Most experts believe that WASH interventions require many years of sustained efforts before having an ultimate impact on health outcomes. Despite our intentions to roll out the intervention as quickly as possible, setting up a WASH program takes time, and our intervention was not fully implemented until month 12. This leaves two years of follow-up time in WUHA I, which may be insufficient to see the full impact of a WASH intervention. Moreover, despite 8 annual rounds of mass azithromycin, the prevalence of ocular chlamydia started at a high level in WUHA I. WASH interventions may reduce transmission of infection, but likely do not clear existing infections, and therefore it may be more difficult to show an effect of WASH at a high prevalence of chlamydia. In WUHA II we will administer annual mass azithromycin all 40 clusters, which should greatly reduce the prevalence of ocular chlamydia, and perhaps make it easier to find a difference between the 20 WASH + azithromycin clusters and 20 clusters that receive antibiotics alone.

4.1 Study Area

SWIFT is being conducted in three woredas of the WagHemra zone of Amhara, Ethiopia: Gazgibella, Sekota, and Sekota Town (Figure 2). In Ethiopia, a woreda is a government-defined administrative subdivision that is in turn comprised of kebeles. WagHemra is arid and mountainous, and was chosen

Figure 2: SWIFT I/II study site

based on trachoma impact surveys performed by the Carter Center that determined the area had a high prevalence of trachoma and a low prevalence of several WASH indicators.

4.2 Eligibility Requirements

Intervention Eligibility: School districts were eligible for inclusion in WUHA I if a site could be identified for water point construction and at least 5 rounds of mass azithromycin distributions had been performed. School districts were excluded if they were in an urban area, since urban communities have better access to water and sanitation and have less trachoma.[25,26] School districts were also excluded if they required more than 1 day of travel to access. Forty communities met the eligibility criteria and were included. All 40 communities enrolled in WUHA I will also be enrolled in WUHA II.

4.2.1 Monitoring Eligibility:

Population-based sample:

For the population-based sample, we will monitor the following 3 groups: (1) a random sample of 30 children aged 0-5 years, (2) a random sample of 30 children aged 6-9 years, and (3) a random sample of 30 individuals 10 years (Table 1). We will monitor the 0-5 and 6-9 year age groups annually and the adult group at the start and end of the study period. Eligibility for each of these groups will be based on the previous census; a new random sample will be selected each visit. All age strata will receive conjunctival photography and swabbing, and children under 10 will have a face photograph taken and a dried blood spot collected. In addition, a random sample of 15 children aged 0-5 years will undergo nasopharyngeal swabbing to assess for macrolide resistance. We monitor all three age strata because information on clinically active trachoma and chlamydial infection in the older age groups will be helpful when comparing this study to previous studies and for future attempts to use the data in mathematical models. In addition, the ability to report the prevalence of clinically active trachoma in 1-9 year-olds will help put the results of the trial into context for trachoma programs, which base treatment decisions on this metric.

Table 1. Age strata and tests to be performed in each study community, population-based sample

Pop.	No.	Eye photo	Eye swab	Face photo	NP swab	Blood spot
0-5 y	30	✔	✔†	✔	✔‡	✔
6-9 y	30	✔	✔	✔		
≥ 10 y	30	✔	✔			

†Primary outcome (chlamydial infection in 0-5y)

‡Nasopharyngeal swab, collected on a random sample of 15 children

Longitudinal cohort:

The longitudinal cohort will consist of the random sample of children aged 0-5 years at month 0, followed annually until month 84. Children who are under 12 months and are selected for the random sample will be added to this longitudinal population, to be followed until the conclusion of the trial. Note that some of the children in the longitudinal cohort will also be selected as part of a population-based sample and so may have additional tests performed. Children in the longitudinal sample will have conjunctival swabs, conjunctival photographs, and dried blood spots performed at each annual study visit.

4.3 Randomization

Randomization unit

The randomization unit for this trial is the primary school district. We included 40 primary school districts from the 3 woredas. We chose the school district for two reasons. First, school children in Ethiopia have a considerable burden of trachoma, so units smaller than a school could be subject to contamination. Second, we wished to incorporate school-based hygiene promotion in the intervention, which requires a randomization unit of school district or larger. Within each school district, the best site for water point development was identified via geohydrologic survey, and the cluster of households within 1.5km of this potential water point was designated to receive the full package of WASH interventions as well as annual monitoring visits for trachoma. We call these households a study cluster.

Selection of clusters

In the spring of 2015, collaborators from Catholic Relief Services in Ethiopia performed a geohydrologic survey of the study area and identified sites that could potentially be developed as protected springs, hand dug wells, or shallow boreholes. We chose the best site from each of 40 school districts for inclusion in the trial, and censused all households within 1.5km of the potential water site.

Intervention randomization

We randomized communities to the WASH arm or delayed WASH arm after the baseline examinations in order to limit the possibility of differential outcome assessment at the baseline visit. The randomization sequence was generated by the trial biostatistician in San Francisco using a random number generator, without stratification or blocking.

4.3.1 Contamination

Cluster-randomized trials are subject to contamination between clusters, which could weaken the effect of the study intervention. We reduce the chances of contamination by studying only a single cluster of households from a relatively large geographic area (i.e., school district), essentially creating a buffer zone around each study cluster. The chances of contamination are further reduced for household-delivered interventions since they are provided only to households enumerated during the census. We have made no attempt to limit contamination from outside the study, which is theoretically possible since all 40 communities have continued to receive the usual health promotion from the government. The government program consists of two health extension workers per kebele who provide 16 different services, among them health education and promotion of hygiene and sanitation.

4.3.2 Masking

Because of the nature of the interventions, it is not feasible to mask the study participants to treatment allocation. By not masking study participants, there is a chance that they will change their behaviors due to knowledge of their allocated treatment group. In the case of this trial, however, the entire purpose of the interventions is to change behaviors. Although there is a chance that individuals in the non-intervention communities will also improve their hygiene due to knowledge of their allocated treatment group, this is not likely—especially given the difficulty of causing behavior change even under optimal programmatic conditions. Because all interventions occur at the community level, study participants are surrounded by similarly treated individuals and therefore are not reminded of their treatment allocation very often, if at all. Moreover, we monitor WASH outcomes in both the intervention and non-intervention communities in order to assess the impact of the lack of masking on changes in hygiene behavior. We do not inform census workers or outcome assessors of the treatment allocation, although we acknowledge that this information would not be difficult to obtain.

The emphasis of masking efforts for the trial concerns the primary and secondary outcomes, which can easily be masked. All laboratory staff are masked to treatment allocation, as are the conjunctival photograph reviewers. All specimens collected in the field are labeled with a 6-digit random identification number. Photographic graders and laboratory staff have access only to this identification number, and not to any other identifying information for study participants.

We will also assess several key process indicators in a masked fashion from both intervention and control clusters (e.g., annual household survey, structured observations), but others in an unmasked way only in the intervention clusters (spot checks, focus groups).

Chapter 5

Study Interventions

5.1 Mass azithromycin

All individuals ages 6 months and older will be offered a single directly observed dose of oral azithromycin (1g for adults and 20mg/kg for children) annually. Children under 6 months, pregnant women, and those with known macrolide allergies will be offered two tubes of tetracycline ointment to be used twice daily for 6 weeks. Antibiotic coverage will be assessed relative to the census population. Workers will return to households until they distribute antibiotics to at least 80% of individuals. In previous studies, we have routinely achieved >85% antibiotic coverage.[27,28]

5.2 WASH Interventions

A comprehensive WASH package was rolled out over the first year of the study. The Carter Center Ethiopia was the principal implementing partner. The interventions were informed by focus group discussions held in the study area as well as nearby areas. Two study staff known as hygiene cluster coordinators are responsible for ensuring the fidelity of the intervention. These individuals both had prior experience implementing WASH interventions for local nongovernmental organizations, and have close ties to local governmental health, water, and school officials. Each element of the package is described in more detail below:

5.2.1 Water

- **Community water point:** We worked with Catholic Relief Services (CRS) to install water points. CRS is a nongovernmental organization with extensive experience constructing water points in Ethiopia. CRS performed a geohydrologic survey and coordinated water point construction in each of the 20 WASH communities with the help of the local Ethiopian nongovernmental organization Water Action. Of the 20 water points, 13 were spring developments, 4 were hand dug wells, and

Figure 3: Household Wash Station

3 were shallow boreholes.

- **Water committee:** Interactive community participation is important for the successful implementation of a water point.[29] The Carter Center study coordinator and Catholic Relief Services worked with kebele leaders to identify a 5-person water committee for each water point, with at least 2 female members. This committee developed a plan for maintenance of the water point, including a payment system to subsidize repairs.

- **Household wash station:** During the initial focus group discussions conducted before the study, community members pointed out that one barrier to good face hygiene was the lack of a dedicated wash basin near the household. The ideal household wash station was identified as a jerry can with a faucet, similar to the types of facilities they saw in bigger cities. Based on this information, we provided all households in the intervention communities with such a jerry can (Figure 3), along with a mirror. Households were responsible for building a base for the jerry can out of locally available materials.

5.2.2 Sanitation

- **Latrine promotion:** Current government policies do not allow the provision of building materials or direct assistance for building latrines, and so study activities were limited to latrine education and promotion in WUHA I. Study staff known as hygiene promoters (see below) are responsible for promoting latrine construction and maintenance. Through door-to-door visits, hygiene promoters observe for the presence and functionality of latrines, and provide education about the value of a

latrine as well as practical tips for construction. We continue to have discussions with local and regional government officials, we are piloting cost-sharing of concrete latrine slabs for households that are willing to pay for part of the slab purchase.

5.2.3 Hygiene

- **Targeted behaviors:** Hygiene education is most effective when confined to a few key messages, repeated in many different settings.[30] Although the WASH package could have a positive impact on many health outcomes (e.g., diarrhea, soil-transmitted helminths, respiratory infections), we focus on two behaviors that are most biologically plausible to have the greatest impact on trachoma: (1) using soap and water to wash a child's face twice per day, and (2) consistently using latrines for defecation. Our messaging (e.g., times of day to wash the face, inclusion of soap, promotion of simple pit latrine, etc.) was based both on our initial focus group discussions and local government policies.
- Community-based hygiene promotion:
  - **Household WASH book:** During our initial focus group discussions in the study area, local government health extension workers showed us educational pamphlets for family planning that they found useful for education activities, and recommended that we create something similar for WUHA. What resulted was a 65-page full-color book that provided a roadmap for hygiene education for hygiene promotion workers and health development army members (see Appendix in MOP). The content of the book was based on many focus groups held with local educators, health officials, and water officials. Local artists provided the illustrations. The book was translated into the two languages spoken in the study area. The pictures are meant to be explanatory enough to be utilized independent of text for illiterate community members. The professionally bound book was delivered to each household in the intervention clusters, to be kept for the duration of the study.
  - **Soap distribution:** The importance of soap was evident from the baseline focus group discussions. An American soap manufacturer (SoapBox Soaps, Washington, DC) donated funds to purchase 4 bars of locally produced bar soap to distribute to each household each month. For WUHA II, we are increasing the soap provided to households with more than 4 members, since running out of soap in these larger households was a consistently reported issue in WUHA I. Larger households will receive 1 bar per person per month; households with 4 or fewer members will continue to receive 4 bars per month. The soap is purchased in Addis Ababa and transported by truck to the study site, where it is stored in one of two custom-built storage sheds in the study area.
  - **Hygiene promotion workers (HPWs):** Twelve people were initially hired as HPWs to live in the study communities and perform door-to-door education activities. The HPWs use the WUHA household WASH book as a basis for interactive teaching, with the goal of covering all topics of the book with each household over the study period. The HPWs also use a standardized form to make hygiene observations at each household (e.g., the presence and use of latrines and wash stations), and use the results to focus their activities. HPWs report to the study hygiene cluster coordinators. As part of SWIFT II, we increased the number of

Figure 4: Household Wash Book

HPWs to 20, so that one HPW is assigned to each cluster, since this aspect of the intervention has proved to be quite popular among participants.

- **Non-study personnel**:
  - *Health Development Army (HDA):* This is a government-run program in which communities are divided into one-to-five networks, with one HDA household in charge of health education for five other households. HDA members receive government training in various health topics, including hygiene. For WUHA, we perform annual supplementary training sessions on hygiene and encourage the HDA members to promote hygiene and sanitation messages to the 5 other households in their network at least monthly using the WUHA household WASH book. HPWs check-in with the local HDA members regularly.
  - *Priests:* People in this part of Ethiopia are overwhelmingly Christian and devout. We perform an annual training session for all priests in the intervention communities and ask them to promote improved hygiene practices once per month at Sunday services. The HPWs work with the priests to present during these educational sessions.

- **School-based hygiene promotion**
  - Schools provide a convenient site for hygiene promotion and dissemination for several reasons. First, ocular chlamydia is primarily transmitted by children. Second, hygiene behaviors and habits are established in childhood. Third, parents identified school-children as a potential vehicle for hygiene education during focus group discussions.
  - **Curriculum:** We developed a primary school hygiene curriculum in collaboration with The Carter Center and Ethiopian Department of Education that was based off a series of key informant interviews with teachers, principals, and health officials. The curriculum consists of 5 to 6 age-appropriate lesson plans per year for grades 1 through 4. Curriculum development was highly iterative, with many rounds of feedback from all stakeholders as well as thorough pilot-testing in our study area. The curriculum was designed to be interactive and student-centered, and to integrate well with the existing government curriculum.
  - **Teacher training:** a 3-day training was held before the school year in the fall of 2016 and 2017. Refresher trainings are held annually for all new teachers, all 20 principals, and the 20 WASH club leaders. Germ theory and the general principles hygiene are discussed, as are each of the lesson plans.

  - **WASH clubs:** In this part of Ethiopia, children are required to participate in at least one extracurricular activity, and many schools have health or hygiene clubs. For WUHA, we provided training materials for WASH activities (songs, dances, dramas, community engagement activities) to existing WASH club leaders, and worked with principals of all intervention schools to ensure that WASH clubs were formed if they did not exist. All 20 intervention schools have active WASH clubs.

- **Hygiene cluster coordinator and hygiene promotion worker trainings** After their selection, the hygiene cluster coordinators and hygiene promotion workers (HPWs) are trained at an intensive workshop led by the Proctor Foundation and Carter Center. We review basic information about trachoma and its transmission, as well as the benefits of the WASH strategy for preventing transmission of trachoma. Although not the target of the current intervention, we also include information about diarrhea prevention. We make training as participatory as possible using various methodologies, including lectures, case studies, role play, and games. We train the hygiene cluster coordinators and HPWs using a "train the trainer" paradigm, since they are in turn be responsible for leading workshops in the study communities. We use a "teach-back" model, where the hygiene cluster coordinator/HPW is asked to convey health promotion information back to the study coordinators, and the study coordinators provide feedback.47 Before being certified, the hygiene cluster coordinators and HPWs have to pass a written and oral examination in the local language, conducted by the Carter study coordinator.

Chapter 6

Intervention Monitoring

We designed several ways to monitor the fidelity of the intervention.

6.1 Spot Checks

The study hygiene cluster coordinators conduct several annual rounds of spot checks in intervention clusters. At each spot check, the hygiene cluster coordinator assesses the usability of the well and checks in with the school teachers in the cluster to review their implementation of the hygiene curriculum. The hygiene cluster coordinator also visits a random sample of 8 households with pre-school children and documents the presence of a wash station and its functionality (e.g., presence of water in the container and soap), the presence of a latrine and its functionality (e.g., whether walls and a roof are present), and evidence for latrine use (e.g., trodden latrine path, fresh feces in the pit). The content of these structured spot checks is based on our experience in Ethiopia and the experience of others who have conducted similar surveys.[31,32] The hygiene cluster coordinator enters all data into a smartphone using an offline REDCap mobile application, and then syncs the data later that evening. Electronic data capture is important, since it allows us to analyze and share the data more rapidly. As shown in Figure 5, the spot check data are visualized as a heat map in order to easily identify the hygiene gaps in each community. The hygiene cluster coordinators then convey this information to the hygiene promotion workers during their monthly phone call and

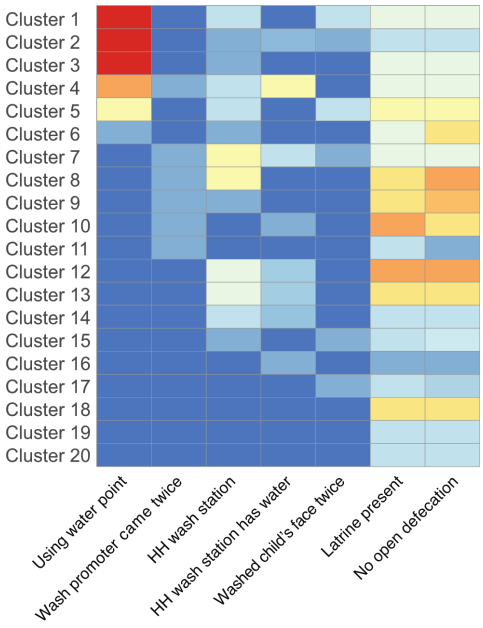


Figure 5: Spot check example. March 2017 spot check of 20 WASH communities, visualized as a heat map (blue=100% of households; red=0%).

they brainstorm ways to improve the deficiencies.

6.2 Household survey at annual census

We perform an annual household survey in a random sample of 33% of households during the census. Census workers are not informed of the study purpose or the randomization allocation. The survey questions capture both self-reported hygiene behaviors as well as objective observations and has been crucial for understanding the uptake of the intervention and for guiding remediation efforts. Figure 6 shows the results from key survey items from the first three censuses (December 2015, 2016, and 2017). The month 24 results show that communities randomized to the WASH intervention are more likely to have a household WASH station and latrine, and household members from WASH communities are more likely to report having washed their face and used the latrine in the past day. Behavior changes in the WASH arm were most evident between the month 12 and 24 visits, which is likely due to two main reasons. First, hygiene behaviors are notoriously difficult to change, and hygiene interventions are thought to require long periods of time before they can influence behavior. Second, our WASH intervention has many components, and was not fully implemented until the month 12 visit, meaning that the period of time between month 12 and 24 is likely a more accurate reflection of its impact. Given how difficult it is to change hygiene behaviors, these results suggest a real and marked difference between the two arms, and provide strong evidence that our intervention is being implemented as intended.

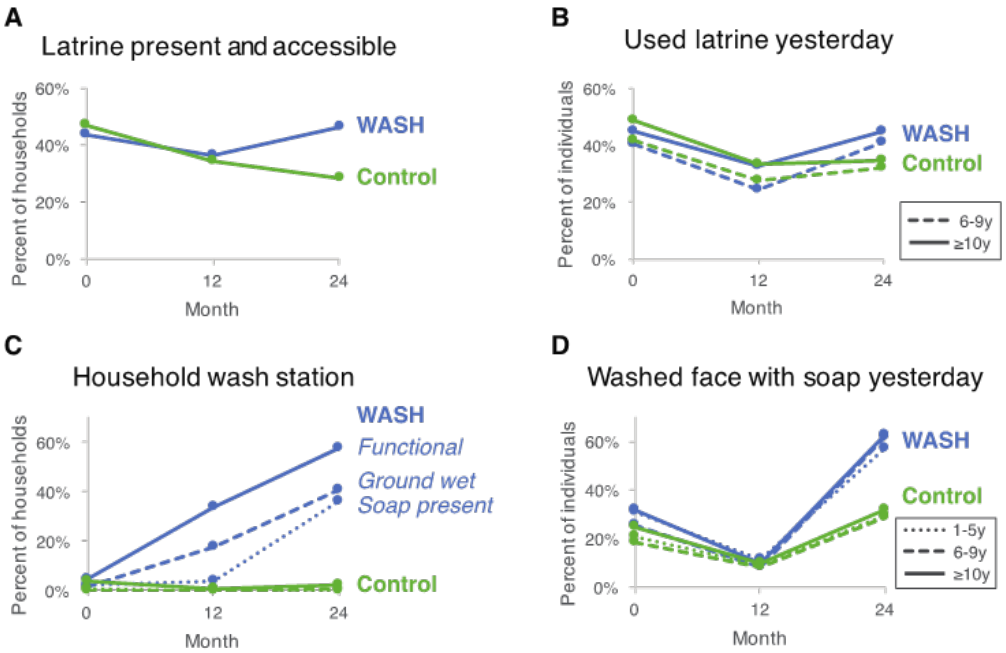


Figure 6: Household survey at month 0, 12, and 24. The survey was performed on a random 33% of households from 20 WASH clusters and 20 control clusters during the annual census.

6.3 Focus Group Discussions

Hygiene promotion must take the local context into account. Barriers to changing hygiene practices and factors that motivate changing behavior are site-specific. We build off previous successful hygiene promotion efforts that have relied on formative research and community participation to guide health messaging priorities.[33–35]

The study team will hold focus groups periodically throughout the trial. The team will choose a representative sample of communities and they will use a discussion guide to lead the focus groups in a discussion about barriers and motivations for hygiene practices, especially as they relate to face washing and latrine use. Sessions will last no more than 2 hours. We will target all appropriate stakeholders. These will include at least the following groups:

- (1) Health Development Army
- (2) Community Leaders
- (3) Strong WASH performers
- (4) Poor WASH performers: Poor WASH performers are a group of people that will be identified by HPWs as those individuals who have poor hygiene behaviors. This may mean that they do not own or use a latrine, that they do not have a wash station at the household, that their compound is not kept clean, etc.

The focus groups are analyzed during the study visit during which they are conducted in order to inform the WASH intervention in a timely manner.

For example, we performed a round of focus group discussions in 5 intervention communities in June 2017 to assess the impact of the intervention and also to help guide our intervention moving forward. We learned that community members highly valued the hygiene promotion workers and soap distribution. We probed for reasons that latrine construction was low; participants reported that besides people's habits being difficult to change, the costs of latrine construction were a major barrier. We also learned that households appreciated the WASH book, that it was regularly used during home visits, and that the illustrations were easily understandable.

6.4 Structured observation

Although we will capture the presence of hardware (e.g., improved water points, latrines, washing stations) during the annual census, this trial also requires detailed assessment of hygiene behaviors (e.g., face-washing and latrine use). Moreover, these assessments must occur in both treatment groups in order to fully assess the impact of the WASH package. Various methodologies exist to measure hygiene behaviors, including qualitative techniques, surveys, and structured observation.[33,36,37] Each has limitations. For example, structured observations are vulnerable to reactivity, wherein those being observed

change their behaviors simply from the process of being observed.[38] Although it cannot completely be eliminated, we tried to limit reactivity by first performing a nutrition survey in 20 households per community, and telling observed households that we were observing their nutrition as opposed to their hygiene.

Structured observation has been used routinely and is well accepted by the WASH sector. We hired and trained a team of local individuals to perform 22 hour structured observations of face-washing and latrine behaviors in both the WASH and control communities during SWIFT I, and we aim to do the same during SWIFT II. By performing the observations in both intervention and control communities, we will be able to better determine whether hygiene behaviors are significantly different in the WASH clusters. We will also use this information to improve the intervention. We performed the SWIFT I structured observations in September 2017. They were conducted in 5 randomly selected households from each of the 20 intervention and 20 control communities. Workers slept overnight at the observed household so that they could observe face-washing and latrine behaviors when they are most likely to occur: in the evening and early morning. The data was double data-entered in Ethiopia.

Chapter 7

Census

We will perform a house-to-house census of the study area in November-December of each year, approximately one month before the exams. The first census took place in November 2015 (Table 2). We hire local workers to perform the census and performed all data collection using a custom-built mobile application. Census workers record the name, sex, and age of each household member, the primary school(s) that any children attend, and the GPS coordinates of the house. In a random one-third of households, we perform a household survey to gauge self-reported socioeconomic status, access to water, and hygiene behaviors, and also perform observations of household latrines and wash stations.

Table 2. Baseline census characteristics.

	Median (Interquartile range)	
Characteristic	WASH N=20	Control N=20
Households	79 (54-119)	88 (68-133)
Individuals		
0-5y	59 (41-80)	68 (51-84)
6-9y	44 (26-58)	52 (34-69)
≥10y	229 (150-282)	276 (210-407)

7.1 Equipment

Each census worker is given the following equipment:

- Tablet computer: Huawei Ascend mate 2 or Mi A2 (one)
- Battery Pack: Anker dual port charger (one)

- Charging cord (from charger to electrical socket); (one)
- Connecting cord (from computer to charger); (one)
- Protective case (one)
- Sealable canvas bag to hold all equipment (one)

7.2 Mobile Application

A custom-made software application, which runs on the Android platform, will be used for data collection in the study. Census workers will enter all data directly into the application. The application is capable of using Amharic letters, so census workers will type the names of all study participants in Amharic.

Census workers will be trained how to upload and download data. At the initial census, there was no pre-existing data so downloading is unnecessary, but census workers should upload each night when they return from the field. At a follow-up census, the pre-existing data from the most recent census will be downloaded in the morning before going to the field. The data will be updated in the field, and then uploaded when the teams return back to town at night.

The data will be uploaded to a secure online server on a daily basis so that the study team can monitor census progress in real time.

7.3 Charging Devices

During training, the Carter Center study coordinator will be responsible for charging all devices each night. This will be done at the coordinator's hotel. The study coordinator will be charging 20 devices and 20 chargers per night, so will need to bring sufficient power strips for this task. During the census, the census workers will be responsible for charging their census phones and external batteries at home each night.

7.4 Personnel

The Carter Center will advertise for and hire local census workers. Prospective candidates should be familiar with technology, and quick to learn new tasks. Candidates should also be able to walk long distances.

7.5 Training and Monitoring

The study coordinators from the Proctor Foundation and TCCE will run a 3-day training workshop at the woreda center for all census workers to train them in the following topics:

- General use of the tablet computers
- Entering data into the mobile application
- Acquiring GPS coordinates with the device
- How to ask the census and household survey questions correctly
- Re-charging the device

After the in-class training, census workers are accompanied in the field for training in a practice village and for the first few days of actual data collection to ensure that quality data is consistently collected.

A team leader is selected from each 3-person census team, and that person is responsible for team supervision, ensuring good coverage of the cluster, uploading, and daily charging.

7.6 Census monitoring

The research coordinator will be responsible for monitoring enrollment and loss to follow-up during the census. Salesforce.com will automatically generate a daily enrollment report, which will be emailed to the principal investigator, Ethiopian study coordinator, and hygiene cluster coordinators on a daily basis during active census periods.

Chapter 8

Annual Monitoring Visits

We will perform annual monitoring visits in all study clusters. At each visit, we will collect samples on a subset of the population in the study cluster, as described below.

Note: we will only include the results of an annual monitoring visit in the final analysis if they occur three months or more after a round of mass drug administration. Mass drug administration would be expected to greatly reduce infection in both treatment arms, which could potentially mask the effect of the hygiene intervention.

8.1 Sampling Strategies

8.1.1 Population-Based Sample

The population-based sample will consist of an age-stratified cross-sectional sample at each monitoring visit (Table 3). Eligibility will be based on the most recent census (which will be completed annually several weeks before monitoring visits). We will sample a separate cross-section of individuals at each monitoring visit, so individuals may or may not be sampled at successive visits . We employ this strategy in order to be able to obtain an unbiased population-based estimate of infection and other outcomes at each visit of the trial.

8.1.2 Longitudinal Sample

For outcomes such as anthropometry, clinically active trachoma, and facial cleanliness, we are interested in assessing changes within an individual. Therefore, we will also employ a longitudinal sampling strategy for these outcomes. Specifically, we will assess longitudinally the entire cohort of children that is aged 0-5-years at baseline. As children are born, we want to capture them in this longitudinal sample as well. To operationalize this, 0-12 month old children who are included in the population-based sample each year are added into this longitudinal sample in the following year and going forward. For the outcomes of

face photographs, conjunctival photographs, dried blood spots, we will monitor the 0-5-year-old cohort from baseline over the course of the study visits in both SWIFT I and II. We will monitor anthropometry in the longitudinal sample during SWIFT I.

Table 3: The number of individuals in each age stratum will depend on the particular outcome:

	WASH trial		
Test	**0-5 years**	**6-9 years⁺**	**= 10 years⁺**
Conjunctival swabs	30⁺	30⁺	30[1]
Eye exam/photo	30*	30	30[1]
Face photo	30*	30	
Nasopharyngeal swab	15⁺		
Dried blood spot	30*		
Anthropometry	30*		

± 2 swabs per participant (2 right eye)
*Longitudinal sample
⁺ Random sample, only done and baseline and endline
[1]This group will only be examined at 84 months

8.2 Preparation

8.2.1 Registration Lists

Registration lists must be made prior to going to the field. This is done in Salesforce.com, keeping the following points in mind:

- Make sure the census is complete. Check in the app to make sure all households have been completed.
- Make sure the sub-phase is set to Examination on the Geographic Work Unit object.
- Make sure you are logged in with an account that is set to the Ethiopian timezone.
- Navigate to the Geographic Work Unit and click on Create Morbidity Study.
- Generate a PDF to give to the mobilizer(s) for participant recruitment.
- Before going to the field, download the registration lists on each tablet/smartphone. This requires internet connectivity.

Supplemental Children: If the desired 80% coverage is not reached after 2 visits to a cluster, the research coordinator or the study coordinator will add supplemental children. This is done in the Morbidity Exam Phase tab on SFDC.

8.3 Training

8.3.1 Pre-Monitoring Visit Training

All new members of the San Francisco-based team will complete a training at the Proctor Foundation before departing for Ethiopia. The training will focus on 1) travel to Ethiopia, 2) study background and rationale, 3) how to perform the clinical exam and 4) how to perform all collection procedures and protocols in order to standardize procedures among graders and tubers. The training will be led by the Proctor research coordinator, with input from the laboratory technician on proper collection and data recording techniques if necessary. The principal investigator or co-investigator will offer in-depth training on performing the clinical exam while in Ethiopia.

8.3.2 In-Country Training

Before the start of the study, local health workers will be selected to complete study fieldwork and will undergo a series of rigorous trainings and certification in clinical grading and swab collection.

At the start of each successive collection phase, examination team members (nurses, lab workers and other health workers from the Ministry of Health as well as general workers from WagHimra) will participate in four-day training in WagHimra. Each team (registration, anthropometry, swabbing, and blood collection) will participate in a role specific trainings and practice during these classroom days. The first 1-2 days of fieldwork are practice training days for all specimen collectors and graders on the data collection teams. The principal investigator and study coordinators will offer on-going training and supervision to all specimen collectors and graders in the field throughout the SWIFT study monitoring visits.

8.4 Exam Team

8.4.1 Mobilizers

The Carter Center study coordinator will hire a community member from each monitored community to mobilize the selected children and ensure a high turnout for the monitoring visit. This person will also select the location for the monitoring visit set-up within the community and remove any study specific hygiene materials that could unmask the outcome assessment.

8.4.2 Outcome Assessors

In collaboration with woreda health officials, the Carter Center study coordinator will hire lab workers, nurses, and general workers from WagHimra to perform all outcome assessments. These workers will receive a 4-day training session at the beginning of the monitoring visit to review all study procedures.

They will be masked to treatment allocation.

8.5 Registration Station

8.5.1 Personnel

One registration worker is needed per exam team. This person must be familiar with the mobile data collection application, comfortable using a tablet/smartphone, and speak the local language. The registration person will be recruited from the general worker population in WagHimra.

8.5.2 Procedures

The Registration Station is always the first station that study participants should visit. It should be placed in a visible area. The registration worker performs the following:

- Download the registration list in the mobile application; normally this will be done before the team arrives in the field, in a location with good internet connectivity.
- Identify the participant on the list of potential participants from the mobile application, and click the name to enter the participant's registration page.
- Verify demographic information (name, age, sex, mother, father).
- Note the background color of the participant's name in the mobile application. This color corresponds to the exam station that they must visit.
- Place the next available sticker from the roll of random number stickers onto a bracelet with the color the matches the participant's background color in the application. This number is the participant's registration number.
- Write the participant's full name onto the bracelet.
- Have the participant hold the bracelet below their chin, and press the photo button on the participant's registration data page such that a photograph is taken of the face that also includes the person's name and registration number.
- Fasten the bracelet around the participant's wrist. This should be done fairly tightly, so that the bracelet cannot be easily removed.
- Direct the participant to the next station.

8.6 Swab Station

8.6.1 Personnel

In collaboration with district health officials, the local study coordinator will hire staff from a neighboring district to perform all swabs. The swabbers and tubers will be nurses or health staff. Swabbing staff will receive a 4-day training session at the beginning of the monitoring visit to review all study procedures, and will be masked to treatment allocation. Each swab team will be comprised of 3 people:

- Tuber: must be meticulous and detail-oriented
- Swabber/Grader: must have good clinical skills
- Photographer: must be familiar with the mobile data collection application and be a fast learner

8.6.2 Procedures

Scanning registration code

The photographer will use the bar code scanner function on the mobile application to scan the registration code on the participant's bracelet.

Clinical Photography

Four specific team members will be designated as photographer in order to ensure high quality photographs by an experienced photographer. The photographer will take photographs of the conjunctiva with a handheld Samsung NX digital SLR camera with a macro lens (1:1). Conjunctival photography causes no damage to the eye, is well tolerated by children, and is a standard clinical procedure at UCSF. Clinical photography will be performed before conjunctival swabbing. Photography is incorporated in the mobile application; photographs will be taken under a specific child's record and therefore automatically associated with the child's unique identifier. Photographs are stored on the memory card in the camera. A compressed version of the photograph is synchronized to the study database along with the other data. The full-size version of the photograph will be transferred to an external hard drive each week. Batteries are charged each night; each camera bag has at least 1 spare external charger.

Photography Protocol

Equipment needed

- Camera: Samsung NX, which has the advantage of running on Android, and therefore has seamless integration with the mobile application

- Miscellaneous equipment: Extra battery, media card reader, lens filter, extra media card

Camera Setup

- Lens: Samsung 60mm f/2.8 macro lens
- Settings: The photographer does not need to adjust the camera settings because the settings are automatically set by the mobile application according to the following parameters:
  - White balance- automatic
  - Aperture Priority
  - F-stop: f/32
  - ISO 400
  - Manual focus
  - Flash: on (fill-in)
  - EV (brightness): 0

Conjunctiva Photograph Procedure

- Place participant into position that will allow maximum stability; standing, sitting or "head-clamp" position. Employing a village volunteer to help is very useful.
- Extend lens fully, to the 1:1 position
- Image is brought into focus by changing the working distance, not by turning the lens. This is because the lens is fixed in its manual setting. The working distance is approximately 20 cm from the eye with our current settings
- Take minimum of 2 photos. If there is any doubt of the quality of the photo while the patient is in position it is better to continue to take more photographs before the patient is allowed to leave.
- Check photos before allowing child to leave. If they are not acceptable, repeat procedure. Only stop if the patient or guardian requests that we stop, or if is deemed impossible, even with further attempts.
- If the child cannot be photographed for some reason, it does not affect the eligibility of the child. A notation is made on the child's record of why the photograph cannot be taken, but no replacement is sought. This is expected to be a rare event.

Face Photograph Procedure

Face photographs are taken with the same camera and lens, using settings defined by the mobile application (same as above, except an F-stop of f/11). The photographer stands approximately 1-2 meters from the participant and moves the lens until the face fills the frame of the photograph, with only a

small amount of space between the chin/ears/cranium and the edge of the photograph.

Photo Troubleshooting Guide

Note all camera settings are permanently embedded in every photo that is taken and can be viewed with the camera or with any standard commercial photo-viewing software (e.g., Adobe Photoshop; Photomechanic, Nikon View, Canon, etc.)

- Photos too dark: Make sure flash is functioning. If first photos in series are acceptable and then they gradually become less exposed (darker) it might be because the battery is gradually losing power during the session. Note that the flash reaction time increases as the battery power decreases.
- Image not centered: Movement of child or camera (hold child's head between knees of "helper"; stabilize camera with second hand)
- Reflection artifact: Move camera slightly between first and second photos to achieve different angle; gently dab conjunctiva with swab- must be done at periphery to avoid creating inflammation

Conjunctival exam/swab

Gloving

Any hand that will touch a participant's skin must be gloved for the examination. The examiner will put latex gloves on both of their hands prior to touching a participant's skin, and a new pair of gloves will be used for each participant. Instant Hand Sanitizer will be available for hand sanitization.

Equipment

- Swabbing/tubing: tubes, swabs, labels, tube box, cooler, cold packs, metal container for swabs, aprons, garbage bags, absorbent blue pads/chucks, hand sanitizer
- Grading: 2.5X loupes, penlight
- Photography: camera, external battery and cord, lens wipes

Examination Position

- Young children: The examining position to be used in the field for young children will be the classic pediatric ophthalmic examination technique. With the aid of a helper seated directly opposite to the examiner, the child will be positioned with his/her head between the examiner's knees, with the child's face looking upwards toward the examiner. The legs of the child will be straddled across the helper and the arms held gently across the child's chest. Care should be taken to keep the child's eyes above the level of the examiner's knees, in order to properly take the conjunctival swab.

- Older children/adults: For examining adults and older children, the participant should stand or sit facing the seated examiner, such that the participant's eyes are at the examiner's eye-level.

Everting the Upper Eyelid

For all participants, only the right upper eyelid will be examined; the only exception to this is if the right eye is difficult to examine due to eye disease or injury, in which case the left eye will be examined. In order to avoid passing contamination from the child into the eye, once the examiner dons a new pair of gloves, their gloved hands should not be used to position the child. The examiner uses their fingertips to grasp the central portion of the participant's upper lid eyelashes. The upper lid is then everted, using a finger of the examiner's other hand (or the end of a sterile swab) as a fulcrum, positioned superior to the tarsal plate. The everted lid is held in place by the examiner's non-dominant hand holding the eyelashes against the orbital rim, thus keeping the examiner's dominant hand free for swabbing the participant's tarsal conjunctiva later.

Trachoma Grading

The examiner uses the 2.5X magnifying ocular loupes to assess the tarsal conjunctiva of the everted upper right eyelid. The examiner will grade the conjunctiva according to the World Health Organization Simplified Trachoma Grading Scale, as shown in Table 4. A hand-held penlight will be used by the examiner for illumination of the conjunctiva at all times; the only substitute for this is direct sunlight. The examiner tells the trachoma grade to the photographer, who records the grade into the mobile application on the Samsung camera. Before being allowed to grade in the field, each grader must complete a training workshop and pass a photographic test with a kappa of 0.6 or greater relative to a gold standard grade, where the gold standard is the consensus grade from a panel of 3 expert graders,

Table 4: WHO Simplified Trachoma Grading Scale

Definitions of the WHO Simplified Trachoma Grading Scale
TF (Trachomatous Inflammation – Follicular): the presence of five or more follicles in the upper tarsal conjunctiva.
TI (Trachomatous Inflammation – Intense): pronounced inflammatory thickening of the upper tarsal conjunctiva that obscures more than half of the normal deep tarsal vessels.
TS (Trachomatous Scarring): the presence of scarring in the tarsal conjunctiva.
TT (Trachomatous Trichiasis): at least one eyelash rubs on the eyeball.
CO (Corneal Opacity): easily visible corneal opacity over the pupil.
Clinically active trachoma: *defined as either TF or TI. If WHO guidelines recommend that TF alone is the most appropriate sign to follow, then we will easily be able to report this.*

Procedures

In this trial, we will collect 2 swabs. The examiner uses a separate pair of gloves for each study participant. We will store the swabs in microcentrifuge tubes without transport media.

- The tuber opens a Dacron swab in a sterile manner, revealing only the tip of the swab shaft, with the swab head itself remaining sterile deep within the sachet, and makes it available to the swabber to take.
- The swabber passes the Dacron swab firmly over the right everted upper tarsal conjunctiva three times, rotating 120ºC between each pass.
- The tuber holds a tube with the cap open
- The swabber deposits the swab in the tube, and snaps off the shaft of the swab. This should not require counterpressure with the tube cap.
- The tuber firmly screws closed the cap. The tuber checks to make sure that the swab is at the bottom of the tube and taps the tube until the swab falls.
- The tuber places the tube in the tube box within the cooler. Tubes are put in chronological order of swabbing, filling a horizontal row, starting with the top left-hand corner of the box.
- The tuber closes the cooler in between swabs.

Control swabs Two sets of control swabs will be taken in the course of examinations:

- Negative "air" swabs: a swab will be waved in the air, taking care not to contaminate the swab. This will be done near the beginning and end of the work day. The tuber and swabber should collect these swabs when they are still in position with all of their equipment (cooler, tube box, loupes, etc. and personal protective equipment (apron, chuck, gloves) in place. The tube codes are scanned into the mobile application in the designated field.
- Duplicate swabs: The mobile application will randomly select 2 participants per community for a duplicate swab. The swabber will collect duplicate swabs from these participants.

Transportation and Storage of Conjuntival Samples In accordance with the Abbott RealTime protocol, swab samples taken in the field will be transported on ice in a closed, insulated container, and then transfered within 8 hours to a -20°C freezer at a local health center. Swabs will be shipped within 4 weeks to the APHI, where they will be stored at -20°C before being processed.

Nasopharyngeal Specimen Collection

At baseline (month 0, the study's midpoint (month 36), the final study visit (month 84), we will collect nasopharyngeal swabs from 15 randomly selected children aged 0-5 years per community. We selected this age group because pre-school children in sub-Saharan Africa have been shown to have high rates of pneumococcal carriage.[39]

- Immediately after completion of the conjunctival eye swab collection (and if necessary, control swab collection), the tuber will select an NP swab and open the swab sachet in a sterile manner (revealing only the tip of the swab shaft, with the swab head itself remaining sterile deep within the sachet).
- The examiner will remove the NP swab from the sachet and place the tip into the participant's nasopharynx.
- The examiner will quickly rotate the swab 120° three times back and forth, and then remove the swab from the nose.
- The examiner will place the swab in a tube containing 1.0 mL of STGG (skim milk, tryptone, glucose, and glycerin) media. The tuber will cut the handle off using sterile scissors (cleaned with alcohol pads between participants). The tuber will close the cap of the STGG tube with the swab immersed.
- We will keep the swabs on ice in the field and then in a -20°C freezer as described above for the conjunctival swabs. The Amhara Public Health Institute will process the swabs using media selective for Streptococcus pneumoniae, and then test for antibiotic resistance to erythromycin, penicillin, tetracycline, and clindamycin using the Kirby Bauer disc diffusion assay.

Materials for NP Collection

- Nasopharyngeal Swabs: Specimens will be collected using sterile, individually-wrapped pediatric calcium alginate swabs with a malleable plastic swab shaft for patient comfort and safety
- Nasopharyngeal Sample Tubes: All field samples for DNA testing will be collected into sterile 2.0ml microcentrifuge tubes, manufactured by RPI®.
- Cooler Bags with Frozen Ice Packs: Insulated cooler bags will be used to carry samples to and from the field. In addition, frozen gel ice packs designed to thaw slowly will be used to maintain the temperature in the cooler bags during transport.
- -20°C Freezer: A standard -20°C freezer will be used strictly for the storage and freezing of ice packs and samples. This freezer is kept in a locked room on the grounds of the Health Center, which is under 24-hour security guard supervision.
- Latex Gloves: latex examination gloves will be used to perform nasopharyngeal examinations. Each glove will be used once and never shared between participants. Used gloves will be collected in a trash bag and incinerated at the local Health Center incineration facility.

Transportation and Storage of Nasopharyngeal Samples The nasopharyngeal swab samples in skim milk-tryptone-glucose-glycerin (STGG) will be initially stored in the field using Yeti cooler bag filled with Fisher brand ice gel packs and then transferred along with the conjunctival swabs first to a -20 freezer in WagHimra and then to Bahir Dar.

8.7 Anthropometry

Using methods we have already used in several studies in Ethiopia and Niger, we will measure the height, weight, and middle upper arm circumference (MUAC) of the longitudinal cohort of 0-5 year-old children.[8,40] Each index will be measured in triplicate and the median measurement used for analysis. We will use a Shorr Board to measure height, Seca 874 floor scale to measure weight, and standard Ministry of Health distributed MUAC tapes to measure MUAC. Anthropometric measurements in previous studies have had excellent repeatability, and we have found such assessments to be feasible and inexpensive. We will urgently refer any children with severe acute malnutrition, defined as MUAC <12.5cm, to a local health clinic for treatment. The guideline for referral was recently changed from 11cm to 12.5cm by a new government guideline.

8.7.1 Observe Signs of Malnourishment

When the participant presents for examination, first examine him/her for signs of malnourishment:

- Kwashiorkor: look for edema or swelling; thin, sparse, or discolored hair; and skin with discolored patches that may crack and peel.
- Marasmus: look for severe wasting; the appearance of 'skin and bones;' and a face that looks like an old man's.
- Pedal edema: look for swelling due to excess fluid in the foot. Press the child's foot with your thumb. If the foot is swollen and the indentation remains after you press it, the child has edema.

8.7.2 Measuring and Recording Guidelines

Measurements are taken to the nearest 0.1 cm (1.0 mm) for height/length and MUAC, and 0.01 kg for weight.

8.7.3 Measuring Length and Height

A lightweight measuring board will be used to measure the participant's height to the nearest 0.1 cm. Height/length will be assessed with a ShorrBoard (ShorrBoard®, Shorr Productions, LLC, Olney, MD, USA).

Length: If a child is less than 2 years old, measure recumbent length. In the app, select L to indicate that length was measured. A child's length is measured lying down (recumbent).

Height: If a child is aged 2 years or older and able to stand, measure standing height. In the app, select H to indicate that height was measured. Height is measured standing upright.

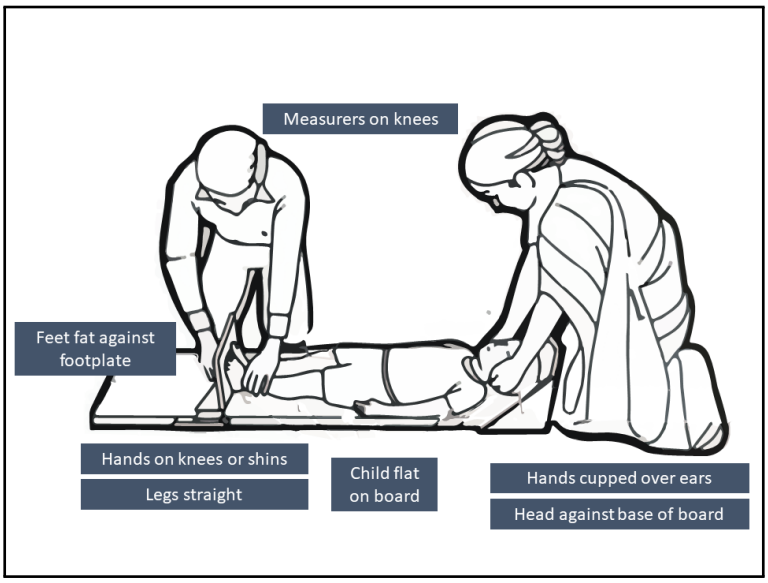
If the child has braids or hair ornaments that will interfere with length/height measurements, remove them if possible. Check that any shoes or socks have also been removed.

Whether measuring length or height, the mother or guardian is needed to help with measurements and to soothe and comfort the child. Explain to the mother the reasons for the measurements, and the steps in the procedure. Answer any questions she might have. Show her and tell her how she can help you. Explain that it is important to keep the child still and calm to obtain the best measurement.

Procedures for Length Measurement

- Cover the length board with a chuck (or another absorbent, disposable material) for hygiene and for the baby's comfort.
- Explain to the mother that she will need to place the baby on the length board and then help to hold the baby's head in place while the measurement is taken. Show her where to stand when placing the baby down (i.e. opposite you, on the side of the length board away from the tape). Also show her where to place the baby's head (against the fixed headboard) so that she can move quickly and surely without distressing the baby.
- When the mother understands your instructions and is ready to assist: Ask her to lay the child on his back with his head against the fixed headboard, compressing the hair.
- Quickly position the head so that an imaginary vertical line from the ear canal to the lower border of the eye socket is perpendicular to the board. (The child's eyes should be looking straight up.) Ask the mother to move behind the headboard and hold the head in this position.
- Speed is important. Standing on the side of the length board where you can see the measuring tape and move the footboard:
  - Check that the child lies straight along the board and does not change position.
  - Shoulders should touch the board, and the spine should not be arched. Ask the mother to inform you if the child arches the back or moves out of position.
  - Hold down the child's legs with the one hand and move the footboard with the other. Apply gentle pressure to the knees to straighten the legs as far as they can go without causing injury or distress. Note: it is not possible to straighten the knees of newborns to the same degree as older children. Their knees are fragile and could be easily injured, so apply only minimum pressure.
  - If a child is extremely agitated and both legs cannot be held in position, measure with one leg in position.
  - While holding the knees, pull the footboard against the child's feet.
- Upon reading the measurement, the examiner will clearly call out the number to the recorder. Record the child's length in centimeters to the last completed 0.1 cm. (1.0 mm).
- Keeping the child in place, release the sliding footboard, and prepare to repeat the measurement. Re-position the child for a second and third measurement.

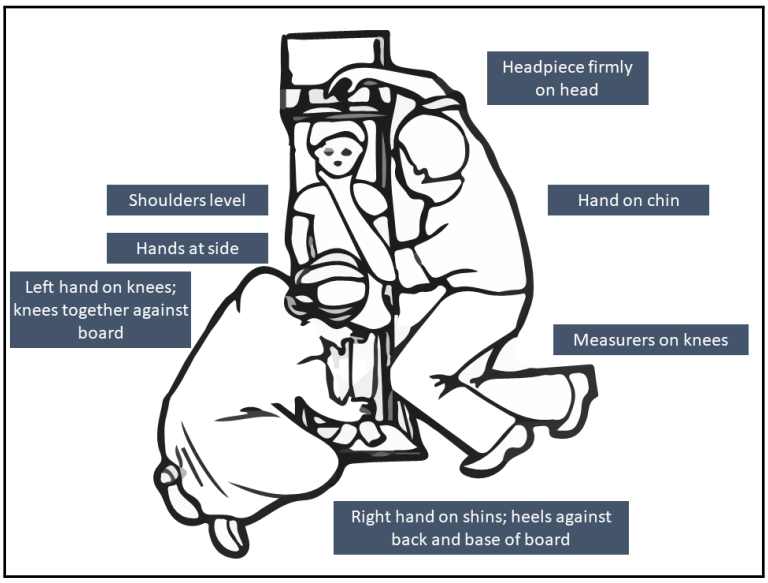
Figure 7: Measuring Length



Procedures for Standing Height Measurement

- Ensure that the height board is on level ground with a wall or tree behind it to support the backboard.
- Working with the mother or another helper, and kneeling/bending in order to be at the level of the child:
  - Help the child stand on the baseboard with the weight of the child evenly distributed on both feet.
  - The heels of the feet are placed together with both heels touching the base of the vertical board.
  - Place the feet pointed slightly outward at a 60-degree angle.
  - The back of the head, shoulder blades, buttocks, calves, and heels should all touch the vertical board. Arms should hang freely by the sides of the body with the palms facing the thighs. Note: Standing with all body parts touching the board may be difficult for some children, in which case, help the child to stand on the board with one or more contact points touching the board.
  - Ask the helper to hold the child's knees and ankles to help keep the legs straight and feet flat, with heels and calves touching the vertical board. Ask her to focus the child's attention, soothe the child as needed, and inform you if the child moves out of position.
  - Position the child's head so that a horizontal line from the ear canal to the lower border of the eye socket runs parallel to the baseboard.
  - Ask the child to inhale deeply and to stand fully erect without altering the position of the heels. If necessary, push gently on the belly to help the child stand to full height.

Figure 8: Measuring standing height



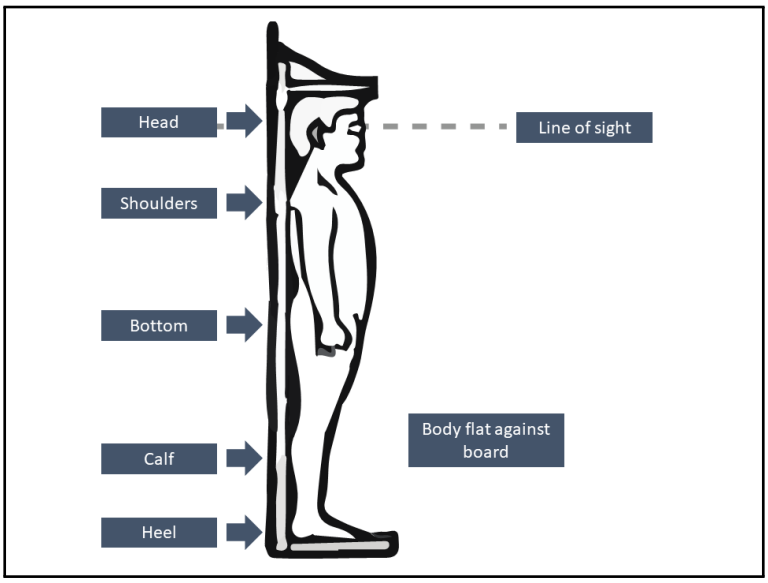
  - Still keeping the head in position, use your other hand to pull down the headboard to rest firmly on top of the head and compress the hair.

- Upon reading the measurement, the examiner will clearly call out the number to the recorder. Record the child's height in centimeters to the last completed 0.1 cm (1.0 mm).
- Keeping the child in place, release the sliding headboard, and prepare to repeat the measurement. Re-position the child for a second and third measurement.

Dismantling the Shorr Board

- Stand the board upright: face the board and step on the base with one foot to keep it stable.
- Slide the head/foot piece into the base of the main board.
- Release the clasp on the back of the extension piece and remove it. Push the clasp FLAT against the extension piece.
- To attach the extension piece to the main board, turn the front of the extension piece inward and place it against the front of the main board. Make sure that all sides of the extension piece are straight and in line with the main board.
- Push on the bolt that is on the back of the extension piece and screw it into the main board.
- Put the board back inside of the carrying case for storage until your next use.

Figure 9: Points of contact



8.7.4 Measuring Weight

The SECA 874 scale or suitable alternative will be used to weigh infants and children to the nearest 0.01 kg (Seca 874 flat floor scale, seca GMBH Co. Kg, Hamburg, Germany). Infants and young children can also be weighed simultaneously with their parent or guardian by the unique "mother-baby" function (parent or guardian is weighed and then the infant or child is weighed while held by the parent).

Explain to the parent that we want to weigh their child to see how he or she is growing. If she has a baby or a child who is unable to stand, s/he will hold the child on the scale. If the child is 2 years or older, the child will be weighed alone. Children should be wearing only light clothing, no shoes, no hair ornaments, and no jewelry. Explain that the child needs to remove outer clothing and shoes in order to obtain an accurate weight. If the baby is wearing a diaper, the diaper should be removed. If any heavy clothes remain on the child, make a note in the notes section of the app.

Procedures

- Remove scale from bag.
- Be sure that the scale is placed on a flat, hard, even surface. All 4 legs of the scale should make contact with the ground surface without wobbling.
- Turn the power on the scale when you are ready to begin weighing the child.
  - Note: When batt appears in the display, you should change the batteries. Remove the old batteries and insert 6 new batteries.
- Press the start key with no load on the scale. The scale is ready for use when it sets to 0.00.

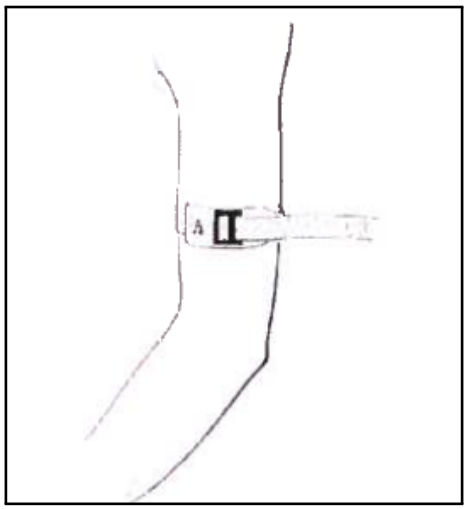
  - Note: if necessary, switch the weight display to KG: hold down the 2 in 1 key for about 3 seconds.

- Weighing the child alone
  - Ask the child to stand in the middle of the scale. Once on the scale, the child must stand still. The HOLD function is automatically activated for weights over 1.5 kg/3.3 lbs. The display flashes until a stable weight has been measured. The display is then frozen until the next weighing operation.
  - Note: If the child jumps on the scale or won't stand still, you will need to use the 2-1 tared weighing procedure instead.
  - The positioner will clearly call out the child's weight to the recorder. Record the child's weight to the nearest 0.01 kg.
  - Have the child step off and back onto the scale
  - The positioner will clearly call out the child's weight to the recorder.
  - Record the child's weight to the nearest 0.01 kg.
  - Repeat one more time

- 2-1 feature: If the child is unable to stand on the scale, you will use the 2 in 1 weighing function (called tared weighing). The 2 in 1 function enables the weight of babies and small children to be determined while an adult holds them.
  - Ask the adult to stand in the middle of the scale without the child. She should remove any long garments, as these can cover the display and also lead to variable measurements.
  - After the mother's weight appears on the display, tell her to remain standing on the scale. Press the 2 in 1 key to activate the function.
  - The scale stores the weight of the adult and the display returns to zero. When 0.00 appears on the display, hand the child to the adult. The scale will determine the weight of the child. Once the value is stable for about 3 seconds, the weight is measured.
  - Note: If a mother is very heavy (e.g. more than 100 kg) and the baby's weight is relatively low (e.g. less than 2.5 kg), the baby's weight may not register on the scale. In such cases, have a lighter person hold the baby on the scale.
  - Repeat the measurement 2 more times. Note that only the baby needs to be removed from the scale; the mother may remain on the scale the entire time.
  - To turn off the 2 in 1 function, press the 2 in 1 key. The 2 in 1 function remains on until you press the 2 in 1 key again, or until the scale switches off automatically.
  - If several children are to be weighed consecutively with the same adult holding the babies, it is important that this person's weight does not change due to a piece of clothing being removed/added.

- The scale automatically turns off after 2-3 minutes

Figure 10: Measuring MUAC



Scale Calibration

We have shown that the weight measurements captured from the Seca 874 do not change over time, even in field conditions.[8] In order to monitor the calibration of the scales over time, each team will weigh a 5 kg test weight at the beginning and end of the day. This measurement will take place at the site of the scale storage so that the weights do not need to be carried to the field.

8.7.5 Measuring MUAC

The child's MUAC will be measured at the midpoint of the right arm between the tip of the shoulder and the tip of the elbow, to the nearest millimeter using non-stretch MUAC tapes used by the Ethiopian Ministry of Health and interpreted according to WHO guidelines[41] .

Procedures for Measuring MUAC

- Have the child stand up straight with the arms relaxed at the sides. The examiner will stand facing the child's right side.
- The measuring tape is placed around the upper arm at the arm's mid-point.
- Wrap the tape around the arm, pulling it to lie flat against the surface of the skin. Be careful not to pull the tape too tightly (to compress the skin).
- Read the number that lies between the two arrows on the window on the MUAC tape. Make sure that the lines inside and outside the window are completely aligned (that the line inside the window is not tilted). Upon measuring, the examiner will clearly call out the number to the recorder. Record to the nearest 0.1 cm (0.1 cm = 1 mm).

8.7.6 Materials for Anthropometry

- ShorrBoard
- Seca scale
- MUAC strips (2)
- Chucks
- Marker
- Trash bags
- Extra set of AA batteries (6)

8.8 Dried Blood Spots

The gold standard microbiological test for detecting ocular chlamydia infection is a nucleic acid amplification test of a conjunctival swab. However, new serological tests being developed by the United States Centers for Disease Control and Prevention (CDC) are promising diagnostic modalities for monitoring of trachoma elimination. A positive test indicates a previous exposure to chlamydia as opposed to current infection; antibodies remain detectable even after a mass azithromycin treatment.[42] Preliminary testing in a hypoendemic setting in Tanzania has shown that serologic results in 1-3 year-old children is correlated with the prevalence of ocular chlamydia infection in a community, suggesting that the serology results in young children may provide information about the level of transmission.[43] Current serologic methods are expensive, but the CDC is currently developing a much simpler and less expensive lateral flow assay (LFA) for use with eluted dried blood spots.[44]

In a setting where ocular chlamydia transmission has been eliminated, the youngest children will not be exposed to chlamydia and therefore should not have a positive serological test. The serological test in young children may therefore be a sensitive measure of chlamydial transmission within a community. Furthermore, we will use the blood spots to test antibody responses to other diseases of interest including helminths, intestinal bacterial pathogens, and intestinal protozoal pathogens. These tests are still being developed, but we anticipate that they will have been optimized by the time of the conclusion of this trial. Therefore, we will also collect dried blood spots from all children aged 0-9 years in the population-based sample each year. We will lance the index finger of each child and express 4-5 dried blood spots onto a TropBio filter paper. Filter papers will be labeled with a random number identification sticker and stored in the -20 freezer for later processing in Atlanta.

8.8.1 Steps for Blood Spot Collection

- Inform the mother that her child's finger will be pricked to obtain blood to test for trachoma and other diseases. Describe the finger prick procedure, reassure her, and answer all questions. The

blood specimen should be collected as described below to minimize the discomfort of the child and to ensure sufficient blood volume collection.

- Label the filter paper with the corresponding random number sticker.
- Enter the child's information from their bracelet and scan the filter paper's code.
- Put on gloves
- Prepare the disposable lancet. Make sure to use a new disposable lancet for each child. Do not re-use lancets.
- Position the child for the finger stick. Make sure that the child's hand is warm and relaxed. It should be below their heart.
- Hold the child's thumb, middle, or ring finger between your left thumb and finger
- Gently stimulate blood flow towards the puncture site by lightly squeezing the child's thumb or finger from the top of the knuckle towards the fingertip
- Disinfect the finger with alcohol wipe.
- Prick the side of the thumb/fingertip
- For the best blood flow and least pain, prick the side of the thumb/fingertip, not the center.
- Wipe away the first 2 drops of blood with a new sterile cotton ball
- Grip the filter paper on the side without small circles.
- Place a droplet of blood from the finger onto five of the six circles. To fill the cirle, touch the side of the circle. Fill the side whole cirle before moving on to the next cirle. It is more important to completely fill the cirles than to put blood on all 5 cirlces.
- Leave the last circle blank.
- Note: Allow blood to ooze out on its own. Do not squeeze forcefully avoid "milking" as it may dilute the blood with tissue plasma.
- Carefully slide the filter paper onto a pencil to air dry for at least an hour. There should be about 1 cm in between each sample. Secure the pencil into a Styrofoam surface in a cardboard box.
- When dry, place each blood spot paper into an individual small plastic bag while wearing gloves
- Combine all bagged samples in a larger community bag. Add desiccant packets
- Label large bags with:
  - SWIFT "visit number"
  - Cluster name
  - Number of samples collected
  - When were samples collected: (e.g. 19JAN2019)

Risk minimization

It is important to handle all specimens with care to minimize risk of infection.

- Wear gloves: New gloves must be worn for each child.
- Have the child hold the cotton ball. Do not place on table because it can contaminate the table.
- Clean spills: In the event of a blood spill or splash, clean immediately with disinfectant and wipe with absorbent material.
- Disposal of sharps: All lancets must be disposed of properly in sharps containers.

Materials for Blood Collection

- Gloves
- Disposable lancets
- Alcohol wipes
- Cotton balls
- 10% household bleach to clean spills
- Absorbent material for spills (cotton)
- Sharps container
- TropBio circular cards
- Small zip plastic bags
- Desiccant packs
- Large Ziploc bags (handful)
- Materials for drying apparatus: 12 sharpened pencils, Styrofoam, cardboard box

Chapter 9

Data Collection, Management, and Quality Assurance

Similar to our previous studies, all study personnel who assess outcomes will attend an intensive, 2-day training session prior to the annual census, monitoring, and treatment visits. Study personnel will collect all data from the census, monitoring, and treatment visits on tablet computers using a mature custom-built mobile application. Data will be uploaded to a secure Salesforce.com server.

9.1 Data Collection Tools

9.1.1 Tablet Computers

The Proctor Foundation will provide training on operating tablet computers to all data collectors. In our experience, a tablet computer typically retains its charge for a full day's activities, though each team will also have a backup battery pack. Tablets and batteries will be charged overnight in safe, secure houses or a hotel.

9.1.2 Mobile Application

A custom mobile application will be used for all census, monitoring, and treatment visits. For monitoring, this software assigns a unique identification number to each study participant enumerated during the census. The software integrates the monitoring visits with the census information by linking to this unique identifier. We will provide training to use this mobile application for all study activities.

9.1.3 Data Uploading

Data should be uploaded every day that data is collected. Data is uploaded from the devices after logging in online, and navigating to the Upload Status page. Before logging in, the user can choose whether to upload photos or not, by selecting the appropriate box in the Settings page.

Non-photo data:

We will routinely upload data from the tablet computers to Salesforce.com servers using local mobile connection. Non-photo data normally uploads within a few minutes.

Photo data:

Photograph files are larger and can take a longer time to upload. If good mobile connection or wifi is available, photographs can be uploaded in the field. If a good mobile connection or wifi is not available at the field site, then photos can be uploaded from the tablets once the study coordinator returns to the Carter Center Ethiopia headquarters.

9.1.4 Data Downloading

Data should be downloaded whenever the mobile application needs to be populated with pre-existing data. Data is downloaded by logging in online and navigating to the Upload Status page. Key times when data should be downloaded include:

Census:

Before going to the field for a follow-up census, data from the previous census phase should be downloaded for the study cluster being visited that day. By doing this, all previously entered information about individuals and households will appear in the application, and the worker must only update the status fields, but not re-enter names/ages, etc.

Examination:

Before going to the field for any examination, the registration list data must be downloaded on all tablets. This is absolutely crucial for the registration tablet, but also important for the swabbing stations, since the counts of participants from the registration list is used in the algorithm of choosing random participants for duplicate samples.

Treatment:

Before going to the field for any treatment phase, data should be downloaded to the tablet. Only by doing this will the worker know who needs to be treated.

9.2 Data Consistency and Validity

Through range checks, the software ensures to a large extent that there are no inconsistencies or invalid data. The software will create an error file with relevant data such as the form identification, field names and data. Data consistency and errors will also be monitored from Dashboards that are created in Salesforce.com. A Dashboard is a real-time tabulation of data from the trial, and can be used to monitor: enrollment, completion of the phase, number of doses of medication used, number of swabs collected, missing data from the examination phase, and other pertinent information.

9.3 Data Model

The data is organized as a relational database with several objects:

- Geographic Work Unit (GWU; normally the randomization unit)
- Household (HH)
- Person
- Treatment
- Morbidity Exam Phase
- Morbidity Child Details
- Exam Station
- Test Samples
- Conjunctiva

9.3.1 Census

The census data entry uses the GWU, HH, and Person objects. When a new individual or household is entered, this creates a master record that can be changed at any point in the study. At each census visit, at the end of the data collection, the worker must press Census Complete. Pressing this button creates a Snapshot record that records the data as of the time the census is completed.

9.3.2 Exam

After the census for the entire study cluster is performed, a registration list is made by logging into Salesforce and navigating to the GWU object for the study cluster of interest. After setting the sub-phase to Exam, the New Morbidity Study button is clicked. Doing so will create a list of Morbidity Child Details records, and it is these records that are downloaded to the tablet to populate the registration list during the examinations.

During the exam visit, the participant checks into the Registration Station and has a registration code associated with the Morbidity Child Details record. This registration code is then scanned into the tablets of all subsequent stations. The registration code is the way to link each of the measuring stations (e.g., each of the conj. swabs) to the Morbidity Child Details object, and hence, to the Person and Household pages from the census.

9.3.3 Treatment

Before going to the field, the GWU subphase must be changed to treatment. Doing so will cause the treatment assignment and medication icons to appear in the application. The view for the worker is the same as for the census, except icons indicate who needs to be treated and who has been treated. A treatment record is created when the treatment icon is pressed, and the relevant dosing information entered. Note that treatment records belong to a particular Snapshot and are never treated as a Master record.

9.4 Data Collection

The data collection application is available in Amharic and English. Data is collected in Amharic.

9.4.1 Census

The following data is directly input into the mobile application during the census visit:

- Village name, schools/clinics/water sources used by village
- Household identifiers (address, phone, GPS coordinates)
- Consent
- Name, age, and sex of household members
- Location and status of household members
- A hygiene survey is collected during the census for 33% of households

Household numbers and individual numbers for further identification of individuals within each household will be automatically assigned by the application and Salesforce.com. After the household information is entered, the Census Complete button is pressed. Pressing this button creates a snapshot of the data, and is used for registration lists. Data can be updated after pressing Census Complete, but these updates will be applied only the the Master Record, and not to the Snapshot.

9.4.2 Examinations

The following data is directly input into the mobile application during the examination visit:

Registration tablet:

Name, age, sex are pre-populated when the registration list is downloaded; these are confirmed. A registration code is taken from a roll of unique random number stickers and placed on a participant bracelet.

The bracelet color chosen for a participant to wear should correspond to the color on the participant's registration page; this color indicates which stations the participant must visit. The registration code is scanned into the participant's page with a bar code scanner. The worker takes a face photo of the individual to help track them to resolve data discrepancies at a later time; this photo should also include the registration code and the name of the participant.

Swab tablet/camera

The photographer is responsible for all data entry at the Swab Station. The main menu for the swab station on the mobile application has various colors that correspond to the colors of bracelets being used for the study. It is important to select the correct tab based on the participant being examined (e.g., the color tab that matches the color bracelet). The different colored tabs correspond to different complements of tests, so if the incorrect color is pressed, then that participant will not have the correct tests performed. After pressing the color tab, the participant's bracelet is scanned with a bar code scanner. A list of buttons with the required tests appears; the swab sites on the list should be performed in order, from top to bottom. In each subsequent button, it is possible to enter age and sex information; this should be done only once.

- **Conjunctiva:** The default eye being examined is the right eye, but this can be changed by pressing the Left tab. Photographs are taken. The trachoma grade reported by the Swabber is entered; this is a multi-select field (TF, TI, TT, TS, CO). After the conjunctiva and nasopharyngeal swab(s) are taken, the appropriate tube's random number sticker is scanned with the bar code scanner.
- **Negative controls:** In order to assess the possibility of field contamination of swabs, a negative control swab is performed at the beginning and end of the day and entered into the appropriate field in the application.

- **Positive controls:** For all swabs, a random 5% of participants will be selected by the application to have a duplicate swab. This is evident because a second button appears next to the required duplicate swab after scanning the participant's bar code.

Anthropometry tablet

The anthropometry section functions like the swab station screen, with colors for different groups. The anthropometry data collector will select the correct tab based on the participant being examined (e.g., the color tab that matches the color bracelet). After pressing the color tab, the participant's bracelet is scanned with a bar code scanner. The data collector enters the participant's age and sex information.

Height and weight will be entered in triplicate for each anthropometry study participant. MUAC will be entered once for each study participant The presence or absence of kwashiorkor, marasmus, and pedal edema will be noted for each participant. This data will be entered into the app, which employs data validation.

Blood tablet

The blood section functions like the swab station screen, with colors for different groups. The blood data collector will select the correct tab based on the participant being examined (e.g., the color tab that matches the color bracelet). After pressing the color tab, the participant's bracelet is scanned with a bar code scanner. The data collector enters the participant's age and sex information.

Before the blood spots are taken, the blood spot's random number sticker is scanned with the bar code scanner.

9.4.3 Treatment

During the treatment visit, any data from the census can be updated. following data is directly input into the mobile application during the examination visit:

- Treatment consent
- Medication distributed
- Medication dose

9.5 Costing data

Cost related data will be collected on standardized paper forms. The Proctor Foundation research coordinator and Carter Center study coordinator will be responsible for training the driver/logistics coordinator

and the hygiene cluster coordinators in filling out the costing forms. We will use paper cost forms, with data double-entered into a database by Carter Center personnel in Addis Ababa. During data entry, computers will be automatically backed up once daily.

9.6 Data Security and Storage

Databases at the central site will be stored on an encrypted server in a temperature-controlled locked room at the Proctor site, and off-site backups will be maintained. Backup encryption keys will be maintained off-site in a secure vault. Database procedures will include full transaction logs. The DSMC can make requests to have access to the data at any point during the course of the study.

9.7 Sample Organization and Storage

9.7.1 Sample Organization

The samples are automatically linked to the person-data via the Salesforce.com database and the application that was built for this study. Once samples and sample organization data from Salesforce.com are received by the Amhara Public Health Institute, a staff member uses an electronic data capture system (Brady Code Reader 3.0) to scan the random number stickers on the sample tubes. These are exported as an Excel files and sent to San Francisco for the pooling algorithm.

Samples are organized according to collection time point and study cluster. A detailed sample storage chart is created by the lab staff for each visit and all samples are linked with the study database.

9.7.2 PCR Pooling

Computerized randomization is utilized to prepare samples for pooling.

9.7.3 Sample Storage

Samples will be stored at the Amhara Public Health Institute in -20° freezers. APHI has a protocol to monitor the temperature of the freezers and switch to generator power when needed.The PCR samples are stable if stored at -20°.

After the analyses are completed, all positive samples and a random 10% of negative samples will be banked for future analyses. While samples will be processed in a timely manner, the researchers will not have to contend with sample degradation concerns in relation to time elapsed between data collection and processing. This allows the researchers to continue to utilize the samples for additional future analyses.

Chapter 10

Outcome Assessment and Laboratory Procedures

10.1 Photograph Reading Center Overview

The Reading Center for grading of conjunctival and face photographs will be based at Gondar University. Three graders will independently grade each photograph, masked to treatment allocation. We will choose the consensus or median grade for all outcomes. We will assess inter- and intra-observer agreement with kappa statistics.

10.1.1 Facility/Equipment

A dedicated room with a two computer monitors will be used to grade photographs. Full-screen photographs will be read at 26 inches with the shades drawn and lights off.

10.1.2 Naming Conventions

The Proctor data analyst and program manager will be responsible for organizing the photographs taken during the study. The photos are stored on Salesforce, and they are labeled with an "attachementID" code. The program manager will download the photos and their metadata including the photo code, the phase they were taken during, and the participant's master record that they belong to. The photos will be uploaded to the offline program and linked to their metadata for grading.

10.1.3 Storage

Photographs are stored on the Salesforce.com server and on two separate external hard drives.

Figure 11: Questions for conjunctiva grading

Questions for Conj-grading-swift-036:

Question	Responses
1. **Photo Quality**	• **1** – Good (Default) • **2** – Poor but acceptable • **99** – Ungradable
2. **Photos all of one eye**	• **1** – All of one eye (Default) • **2** – Includes other eyes • **3** – Includes faces
3. **Number of follicles on central tarsal conjunctiva**	• **0** • **1** • **...** • **14** • **15** or more • **99** – Ungradable
4. **Trachoma Inflammation**	

Score	Structure not visible for >50%	Papillae	Description
5	Trunks		Trunks visible ≤ 50%
4	1st branches		Trunks visible > 50%, but not 1st branches
3	2nd branches		1st branch visible > 50%, but not the 2nd branch
2		> 50%	2nd branch visible > 50%, and papillae on > 50%
1		≤ 50%	2nd branch visible > 50%, and papillae on ≤ 50%
0		None	2nd branch visible > 50%, and no papillae
99			Ungradable

5. **Trachoma Scarring**					
	Score	Scarring	Shortening/ Distortion	Involving	Description
	0	None	None		None
	1	Fine, scattered	None		Fine, scattered scars
	2	More severe	None		More severe scarring but NO shortening or distortion
	3	Severe	Yes	≤ 50%	Severe scarring involving ≤ 50% WITH shortening or distortion
	4	Severe	Yes	> 50%	Severe scarring involving > 50% WITH shortening or distortion
	99				Ungradable
6. **Notes**	Include your notes here. (Optional)				

10.1.4 Grading Clinical Conjunctival Photographs

Six ophthalmology residents will grade the photographs at the conclusion of SWIFT I and the conclusion of SWIFT II. They are masked to treatment allocation. We will present graders all photographs taken from a phase for one individual. Graders will grade the set of photographs together for photo quality, follicles, inflammation, scarring according to the grading scale described in figure 11. We chose this grading system because it provides enhanced granularity of grades compared to other grading scales, allowing for detection of more subtle differences in clinical trachoma. Graders will also compare the initial and final photographs of the set and document whether the final photograph has less, more, or equivalent follicles and inflammation compared with the initial photograph. Note that each set of photographs will have between 1 and 4 photographs, depending on how many photographs the photographer took during the monitoring visit. We use photographic grades as the co-primary outcome of the trial since high quality photographs have good agreement with in-field grades, and offer the benefit of a masked outcome.[45,46]

10.1.5 Assessment of Facial Cleanliness

The ophthalmology residents will grade the face photographs for each child. The photos will be organized and uploaded in the same way as the conjunctiva photos. The photo set (1 child's face photos at 1 phase) will be graded on photo quality and the presence or absence of the following items: dust or dirt on the face, food on the face, dry secretions around the eyes, wet secretions around the eyes, dry secretions around the nose, and wet secretions around the nose. The number of flies on the face will also be counted. The grader will be masked to treatment allocation. Note that this is a novel way to determine facial cleanliness, and should result in complete masking of observers.

10.2 Chlamydia trachomatis sample processing

10.2.1 Specimen Collection for Microbiological Tests

Samples will be collected with reference to age, gender, household, and study cluster, but participant names will not be included in laboratory records. Samples will thus not be associated with the individual's name, but with a 5-digit random identification number, masking laboratory personnel and preventing identification of the individuals infected. Lab results will not be available for weeks if not months, typically after the average duration of an ocular chlamydial infection.

10.2.2 APHI Lab: Abbott RealTime Assay

Laboratory testing is the current standard of care for the identification of C.trachomatis infections in the U.S. After collection, all samples will be processed the APHI and will be filled with 1 ml of M4RT media, and tested for C. trachomatis.

Swabs will be processed with the Abbott RealTime assay for Chlamydia trachomatis, using the automated Abbott m2000 System, which is already in use at APHI. The RealTime assay targets the cryptic plasmid of C. trachomatis. The assay has been shown to be highly sensitive and specific for the diagnosis of sexually transmitted C. trachomatis, with sensitivities exceeding that of the nucleic acid amplification test used in most recent trachoma studies (Roche AMPLICOR).[47,48]

10.2.3 Processing of Samples for PCR

Samples are handled as per Abbott RealTime sample processing protocol, a highly sensitive assay that targets the cryptic plasmid of *C. trachomatis*.[48,49] We will perform the following modifications:

1. Samples are boiled for 10 minutes at 100ºC. Boiling of samples is an accepted treatment method to remove substances that may be inhibitory to the PCR amplification process.
2. Samples are pooled.

10.2.4 Procedure for Masking Samples

The PCR results will be recorded according to the random number assigned to the sample, thus masking the lab since the lab workers do not have access to Salesforce in order to link this number with the child who provided the sample. Once the sample is processed, the analyst will then link the sample random number to the child's record on Salesforce to reveal the test results by cluster.

10.2.5 Procedure for Pooling Samples

We will increase the efficiency of chlamydial testing by combining swabs from the same age stratum and same community into pools of 5 random swabs for processing. An internal control will be run with each pool to rule out the possibility of PCR inhibitors. Any inhibitory pools will be re-tested, and if still inhibitory, the swabs will be individually re-tested. If PCR from any pool is equivocal, then all swabs from the pool will be tested individually. While samples will necessarily be diluted in this process, this is not thought to affect the sensitivity of the test.[50] We will test individually all swabs from positive pools in the 0-5 year age stratum and estimate the community prevalence of chlamydial infection as the proportion of positive swabs. We will estimate the community prevalence of infection in the 6-9 and 10 (WUHA) year age strata using maximum likelihood estimation, similar to our previous trials: the number of individual swabs with the maximum likelihood of having resulted in the observed pooled results will be chosen as the estimate for that village.[27,51]

In order to pool the conjunctival samples in the lab, the microbiology lab staff at APHI will assign a new pool ID number for every sample, and samples will be stored at -20°C freezer until PCR testing (if not processed that day).

10.2.6 Quality Control

- A *C. trachomatis*(+) control and a *C. trachomatis*(-) control (targeting 136 base pairs of a pumpkin gene) is included in each test run of the Abbott RealTime assay.
- To test the effect of sample processing, a known positive sample is processed and tested in each test run. (This control is helpful when testing large numbers of negative samples.)
- An internal control intended to identify specimens that contain polymerase inhibitor is run routinely on each sample. The internal control helps identify false negative results.

10.2.7 Calibration

Two levels of calibration must be performed every 6 months:

1. **Optical calibration:** The machine must have optical calibration performed by the manufacturer.
2. **Load calibration:** After the optical calibration has been performed, the PCR technician must perform a standard curve using twofold dilutions of a standard concentration of *C. trachomatis*. This load calibration is valid for 6 months or until the next optical calibration.

10.2.8 Quantification

For every 0-5-year-old who tests positive for chlamydia, we will also run PCR for the beta actin gene on the same sample, in order to normalize the quantity of chlamydial DNA to the amount of the specimen. Quantitative results from the Abbott system are given in terms of a decision cycle (DC) number. We will generate a ratio of the DC number of the chlamydial DNA to the DC number of the beta actin gene and use the resulting ratio as the chlamydial load.

10.2.9 Laboratory Results Reporting

All lab results will be kept in computer files as well as uploaded to Salesforce by the analyst. The principal investigator and the DSMC will be updated regularly on the progress of the lab work throughout the course of the study.

10.2.10 Assessment of ocular chlamydia

Qualitative PCR for chlamydial DNA

Swabs will be processed with the Abbott RealTime assay for Chlamydia trachomatis, using the automated Abbott m2000 System, which is already in use at the APHI. The RealTime assay targets the cryptic

plasmid of C. trachomatis. The assay has been shown to be highly sensitive and specific for the diagnosis of sexually transmitted C. trachomatis, with sensitivities exceeding that of the nucleic acid amplification test used in most recent trachoma studies (Roche AMPLICOR).[48,49] We will increase the efficiency of chlamydial testing by combining swabs from the same age stratum and same community into pools of 5 random swabs for processing. An internal control will be run with each pool to rule out the possibility of PCR inhibitors. Any inhibitory pools will be re-tested, and if still inhibitory, the swabs will be individually re-tested. If PCR from any pool is equivocal, then all swabs from the pool will be tested individually. While samples will necessarily be diluted in this process, 5-pooling has been shown to not significantly affect the sensitivity of the test.[50] We will unpool all positive pools in the 0-5 year age stratum and estimate the community prevalence of chlamydial infection as the proportion of positive swabs. We will estimate the community prevalence of infection in the 6-9 and ≥ 10 strata directly from the pooled swabs using maximum likelihood estimation, similar to our previous trials: the number of individual swabs with the maximum likelihood of having resulted in the observed pooled results will be chosen as the estimate for that village.[27,51] Note that we are currently working with APHI to perform chlamydial testing, so the swab storage, transport, and processing protocols are already in place.

Quantitation of chlamydial load

We will assess the infectious load for all individual specimens from 0-5-year-old children who test positive for chlamydia. Load will be determined relative to a reference housekeeping gene (beta actin) using the Abbott RealTime assay, and expressed as the ratio of chlamydia to this housekeeping gene. We chose beta actin because this is a cytoskeletal structural protein that is ubiquitous and not thought to vary its expression.[52]

10.3 Macrolide resistance testing

10.3.1 Specimen Collection for Macrolide Resistance Testing

Nasopharyngeal samples will be collected with reference to age, gender, household, and cluster, but participant names will not be included in laboratory records. Samples will thus not be associated with the individual's name, but with a 5-digit random identification number, masking laboratory personnel and preventing identification of individuals.

10.3.2 Methods

The nasopharyngeal swab for pneumococcal resistance testing will be transported and processed using standard microbiological techniques at APHI. We will store swabs in STGG medium, keeping swabs on ice in the field and then in the -20°C freezer as described above for the conjunctival swabs. APHI will process the swabs using media selective for Streptococcus pneumoniae, and then test for antibiotic resistance to erythromycin, penicillin, tetracycline, and clindamycin using the Kirby Bauer disc diffusion assay.

10.3.3 Procedure for Masking Samples

The microbiologist and all lab staff will be masked to the cluster and randomization group assignment.

10.3.4 Quality Control

We will follow the standard quality control methods already in place at the laboratory. For example, lab staff will perform positive and negative growth controls for all media, and positive controls for all stains at a pre-determined schedule.

10.3.5 Laboratory Results Reporting

All lab results will be kept in computer files as well as in hard-copy form by the microbiologist.

10.4 Dried blood spot sample processing

We use Trop-Bio filter paper for dried blood spots in SWIFT I/II. Each paper has 6 ears, and we fill 5 ears with a drop of blood.

We ship the dried blood spots to Dr. Diana Martin at the CDC, who processes them for pgp3 and CT694 using the Luminex platform. The Luminex assay results are reported as a quantitative value (median fluorescence intensity minus background; MFI-BG); these results can be dichotomized based on the results of a receiver operating characteristic (ROC) curve from a set of known positives and negatives run on the same bead coupling. Serologic results provide evidence for exposure to chlamydial infection, and thus may be an especially sensitive metric of transmission.

Chapter 11

Treatment

11.1 Mass albendazole distribution

The clusters were initially dewormed with a single mass albendazole distribution to all pre-school aged individuals who are 1 year or older (200mg for children aged 12-23 months and 400mg for individuals 24 months and older). Another albendazole treatment is not scheduled, but school age children receive deworming on a routine basis at the health post.

11.2 Mass azithromycin distribution (MDA)

11.2.1 MDA prior to SWIFT I

The WagHemra zone received 8+ annual rounds of mass azithromycin before the start of the trial, with the most recent treatment occuring 6 months before the baseline visit.

11.2.2 MDA during SWIFT I

No mass azithromycin distributions are planned during SWIFT I.

If the average prevalence of ocular chlamydia in 0-5-year-old children is too high, we will conduct a mass azithromycin treatment immediately after the next scheduled annual monitoring visit. No threshold is set, but consideration will be given to prevalence above 20%.

11.2.3 MDA during SWIFT II

One annual round of antibiotics will be offered as single directly observed dose of oral azithromycin (1g for adults and 20mg/kg for children).[53] Antibiotic coverage will be assessed relative to the census population. Workers will return to households until they distribute antibiotics to at least 80% of individuals. In previous studies, we have routinely achieved >85% antibiotic coverage.[27,28] If MDA takes place within the 3 months prior to examinations, the examination results will not be included in the final analysis.

11.2.4 Mass azithromycin eligibility

During the annual mass treatment, all individuals will be offered a single dose of oral azithromycin, 20mg/kg for children using height-based dosing and 1g for adults or topical tetracycline.[53]

All age groups and sexes are eligible to receive azithromycin, except those contraindicated by Federal Ministry of Health, which currently are:

- Those self-reported as pregnant
- Children under six months old
- Those known to be allergic to azithromycin or macrolides such as erythromycin

These three exceptions will be treated with 2 tubes of topical tetracycline ointment, to be used twice daily for two weeks.

Of note, if the average prevalence of ocular chlamydia in 0-5 year old children is too high, we will consider conducting a second annual mass azithromycin treatment. No threshold is set, but consideration will be given to prevalence above 20%.

11.3 Adherence to Treatment

Adherence to azithromycin treatment will be essentially 100% of those treated since administration of the single dose of antibiotic is directly observed by the distribution team. Adherence to topical tetracycline treatment is less well known.

11.4 Adverse Outcomes and Patient Death

More than 450 million doses of oral azithromycin have now been distributed for trachoma, and reports of serious side effects are essentially non-existent. This may be due in part to minimal surveillance. It also may be due to the fact these are extremely rare with a single dose of azithromycin. In fact, where

carefully monitored, there were actually fewer GI side effects after taking azithromycin. We will create a network to identify any possible post-treatment serious adverse effects.

Azithromycin is generally well-tolerated. The most common side effects of azithromycin and erythromycin are diarrhea or loose stools, nausea, abdominal pain, and vomiting, each of which may occur in fewer than one in twenty persons who receive azithromycin. Rarer side effects include abnormal liver function tests, allergic reactions, and nervousness. Diarrhea due to Clostridium difficile has been rarely reported.

11.4.1 Adverse Outcomes

Non-serious side effects are not uncommon, and serious side effects are possible. The inability of even frequent mass azithromycin distributions to bring about elimination in areas with highly prevalent trachoma suggests that other, non-antibiotic measures may be needed.

The adverse reactions that may occur after taking azithromycin will be explained to individuals prior to enrollment in this study. In the event of an adverse outcome, an alternative treatment for trachoma (e.g. tetracycline ointment) will be administered if the patient needs to continue treatment or for next annual round of MDA. If a patient experiences a serious adverse outcome, they will be advised to alert the health promotion worker or health extension worker, who will help them seek care. This person will in turn inform The Carter Center study coordinator, who will contact the co-investigator at Proctor or at the Carter Center. If, for any reason, they will need further eye care, they will be referred to the nearest health center for examination and treatment, and the most appropriate action will be taken to provide immediate care.

The study team will ensure that appropriate medical care is provided, and that the frequency and severity of adverse events can be assessed. In addition, all adverse events will be recorded and monitored for each individual and reports on adverse events will be made to the DSMC.

11.4.2 Patient Death

The infant mortality rate is quite high in this area of Ethiopia. All deaths since the first census will be carefully recorded during the study. Since the major causes of infant mortality in the area are diarrhea, respiratory infections, and malaria, it is possible that receiving Azithromycin treatment may actually have a positive effect.

In addition, a recent study showing an association between azithromycin and sudden death in adult hospitalized patients.

All death records (if available) will be gathered via the annual census. The incidence of mortality for each study arm will be made available to the DSMC by the statistical analyst.

11.5 Study Medication Description

11.5.1 Zithromax®

Zithromax® is supplied for oral administration as film-coated, modified capsular shaped tablets containing azithromycin dehydrate equivalent to either 250mg or 500mg azithromycin and the following inactive ingredients: dibasic calcium phosphate anhydrous, pregelatinized starch, sodium croscarmellose, magnesium stearate, sodium lauryl sulfate, hydroxypropyl methylcellulose, lactose, titanium dioxide, triacetin and DC red 30 aluminum lake.

Zithromax® for oral suspension is supplied in bottles containing azithromycin dehydrate powder equivalent to 300mg, 600mg, 900mg, or 1200mg azithromycin per bottle and the following inactive ingredients: sucrose; sodium phosphate, tribasic, anhydrous; hydroxypropyl cellulose; xanthan gum; FDC Red 40; and spray dried artificial cherry, crème de vanilla and banana flavors. After constitution, each 5mL of suspension contains 100mg or 200mg of azithromycin.

11.5.2 Albendazole

Albendazole is available in generic form in Ethiopia as a chewable fruit-flavored 400mg tablet.

11.6 Dosage Information

11.6.1 Azithromycin

Azithromycin will be administered as a single dose, in tablet form for adults and in oral suspension form for children. Dosing will be as per the WHO recommendations for treatment of active trachoma:

- 1) Single dose of one gram of azithromycin for adults
- 2) Height-based dosing of children will be acceptable, as per The Carter Center's program—note that this is supported by the WHO PBD group.

Individuals who are either under the age of 6 months, pregnant, or allergic to macrolides/azalides will be treated with 1% tetracycline eye ointment to be applied twice daily to both eyes for a 6 week period. If the appropriate ethical committee in Ethiopia suggests pregnancy tests for self-reported pregnant women, or if the women are unsure of their pregnancy status, then they may be offered an on-site pregnancy test.

11.6.2 Albendazole

Albendazole will be administered to all individuals aged 1 year and up. Children aged 12-23 months will receive half a 400mg tablet (i.e., 200mg) and all other individuals will receive a single 400mg tablet, as per WHO recommendations. Individuals with a known allergy will not receive deworming treatment.

11.7 Alternate Therapies

11.7.1 Alternates to Zithromax®: Tetracycline Ophthalmic Ointment

Tetracycline ophthalmic ointment (1%) is the current standard treatment in Ethiopia for ocular trachoma, and will be distributed to study patients who are not eligible to receive azithromycin.

11.8 Treatment/Monitoring Schedule

As discussed, if the average prevalence of ocular chlamydia in 0-5 year old children is greater than 15% in the treatment arm, we will conduct a mass azithromycin treatment immediately after the next scheduled annual monitoring visit.

11.9 Medication Procurement/Donation

Pfizer, Inc. will provide the donation of Zithromax® (azithromycin), which will be shipped directly to Ethiopia and received by a representative of the Ethiopian Ministry of Health, who will manage the customs process and transport the medication from the port to a storage site. The exemption of duties and taxes will be settled by the Ethiopian Customs Authorities and the Ethiopian Ministry of Health.

11.10 Study Medication Storage and Accountability

11.10.1 Azithromycin

Zithromax® tablets will be stored between 15° to 30°C (59° to 86°F), as recommended by Pfizer. A record of the exact number of tablets distributed and quantity of oral suspension dispensed will be kept by The Carter Center treatment distribution team.

11.10.2 Albendazole

Albendazole tablets will be stored between 20°-25°C, as recommended by the manufacturer.

11.11 Medication Quality Control

Study medication will be stored in The Carter Center project office prior to use. The hygiene cluster coordinators and the study coordinator will regularly check and record the study medication expiry dates. The expiration dates on the medication containers will be strictly monitored and all expired study medicine will be discarded appropriately.

11.12 Checking Antibiotic Coverage

The application allows the users to monitor antibiotic treatment coverage, since treatment is recorded in the application. The application supplies all individuals needing treatment in the app, and treatment is then entered into this page. Treatment coverage can be viewed on Salesforce.com in real time.

The study coordinator will also monitor treatment coverage by visiting the clusters after treatment has occurred and arbitrarily choosing individuals in the cluster to see if they were treated as marked.

Chapter 12

Clinic-Based Case Finding

We will perform clinic-based case finding at all health posts serving the study clusters. Eligible health posts will be identified by interviewing local community leaders during the census and woreda health officials. The cluster coordinators will take photographs of the health post log books. A data entry worker will use the photographs to enter the data into a REDCap form. Data will include age, gender, community, diagnosis, and treatment. Diagnosis of infection or treatment with an antimicrobial will be considered an infectious disease. We will collect data on all individuals who live in a study cluster. We will retrospectively collect data for a full calendar year before the start of the study in order to provide baseline values.

Chapter 13

Costing

We will perform both a short-term and long-term cost effectiveness analysis (CEA) from the societal perspective. For the short-term analysis, we will perform a trial-based cost effectiveness analysis using community-level data from the clinical trial. For the long-term analysis, we will create a decision model to estimate the costs and effectiveness after the conclusion of the study, and determine the cost effectiveness at varying time horizons. For each analysis, we will report the incremental cost effectiveness at a given time point, with a range of uncertainty. The research plan adheres to guidelines for the design and analysis of trial-based and decision analytic cost-effectiveness analyses.[54,55]

13.1 Assessment of costs

13.1.1 Trial-based CEA

We will prospectively collect cost data for all interventions in the WASH clusters during the study period, using the WASHCost costing framework as a guide. WASHCost is a well-accepted methodology in the WASH sector that provides a complete accounting not only of the upfront capital expenses, but also expenditures on capital, maintenance and local support (e.g., community water committees and local governments).

- MDA: The cost of the MDA will be included in the CEA.
- Water points, wash stations, and soap: the study coordinator will record the costs of labor, supervision, materials and supplies, and transport of materials to the community.
- Hygiene promotion: we will record the costs of printed educational materials and supplies for schools, printed materials for workshops and transportation for promotion of WASH intervention
- Salaries and Per Diems: We will include the salaries of the 2 hygiene cluster coordinators and 20 health promotion workers and the per diems given to teachers, kebele leaders, HDAs, and priests during their respective workshops, and also transportation costs for these individuals.

- All Interventions: We will also include as costs the time of community members who volunteer time for one of the interventions (e.g., helping with water point-building or latrine-building, attending workshops or trainings).
- Maintenance: We will make special efforts to enumerate the maintenance costs of the intervention during the second and third year of the trial. Maintenance costs will include the personnel costs of the education and sanitation officer and health promoters, any supplies and equipment needed for maintenance of the water points or latrines, and the time of community members spent at hygiene workshops and repairing latrines or wells.
- Fixed Costs: Finally, we will include a portion of the fixed costs of maintaining the Sekota and headquarters Carter Center offices.
- We will report costs separately for each intervention, as costs per community.

As in previous studies, costing data will be collected at the time the costs are incurred, using standardized forms. We will not include certain protocol-induced costs in the intervention costs. For example, we will not include the costs of the study coordinator, census, or monitoring visits, which are costs imposed by the trial protocol, and would not exist outside the trial. We will, however, keep track of these costs, since they may be useful for secondary analyses.

13.1.2 Decision Model-based CEA

We will use WASHCost's life-cycle costing approach to model the long-term costs of the WASH intervention. We will estimate future costs based on maintenance costs recorded from the second and third year of the trial and using assumptions about the lifetime of the hardware (water points, wash stations, and soap) based on interviews with local service providers. Future costs will be discounted to adjust them to their present value. In modeling long-term costs, we will assume that maintenance-MDA is done at an appropriate interval.

13.2 Assessment of effectiveness

Effectiveness outcomes are discussed elsewhere in this Manual of Procedures, but include:

- Ocular chlamydia (from monitoring visits)
- Mortality (from census)
- Stunting (from anthropometric monitoring)
- All-cause and cause-specific health clinic visits (from clinic-based case finding)

13.3 Distribution of CEA analysis

The CEA analysis will be published in order for it to be useful to governments and other program implementers. If interested parties would like more information about the CEA analysis and data, they will be welcomed to reach out to the Proctor team to further discuss this aspect of the trial.

Chapter 14

Protection of Human Subjects

14.1 Internal Review Board Approval

14.1.1 UCSF Committee on Human Research

The University of California, San Francisco, Committee on Human Research will annually review study protocol for ethical approval. All changes to the protocol will be submitted and approved prior to implementation.

14.1.2 Ethiopian IRB

The study protocol is reviewed annually for ethical approval by the Amhara Region, the Ethiopian National Research Ethics Review Committee (NRERC/MoST), and the Ethiopian FDA (formerly FMHACA). All changes to the protocol will be submitted and approved prior to implementation.

14.1.3 Emory University Institutional Review Board

The Emory University Institutional Review Board will annually review study protocol for ethical approval. All changes to the protocol will be submitted and approved prior to implementation.

14.2 Informed Consent

The head of each kebele will be asked for permission to include the cluster in the study. Additionally, the study will be discussed with all adult family members in the cluster by the participating Carter Center staff members who speak Amharic or the other local language.

At each collection visit, parents/guardians of study participants who are 0-6 years of age will be informed about the possible risks and benefits examination, swabbing, photography, and treatment (if applicable), and asked to give a verbal consent. Young adults and children below 18 years of age, who cannot give consent by law, will be included in the study only following the receipt of verbal informed consent from a parent or guardian. Verbal assent will be obtained by any child over the age of 7. If, at any time, a parent or guardian elects to withdraw themselves or a family member from the study, it will be made clear that they will be offered the same medical treatment outside the study.

14.3 Adequacy of Protection Against Risk

There are several layers of procedures to help minimize study-associated risk to participants.

14.3.1 Conjunctival swabbing

There are minimal risks to the subject who receives conjunctival swabbing for chlamydia. We are aware of no reported complications from this procedure, although a small amount of conjunctival bleeding can occur and a corneal abrasion is possible. Any adverse effects will be treated by the examiners. Ocular examinations will be offered to everyone, even if they choose not to participate in the study. Appropriate ophthalmic care or referral will be provided for any conditions detected during these examinations, regardless of participation in the study.

14.3.2 Nasopharyngeal swabbing

Nasopharyngeal swabbing causes some temporary discomfort but it involves minimal risks without further complications. Any adverse effects, such as nose-bleeds, will be treated immediately by the examiners. Other health care will be provided at no cost to the study participant if necessary, to address a study-related adverse health event.

14.3.3 Clinical photography

Clinical photography of the conjunctiva causes no damage to the eye, is well tolerated by children, and is a standard clinical procedure at UCSF.

14.3.4 Treatment

If treatment is necessary, the risk of antibiotic treatment will be minimized by treating only those who fit in the approved age and inclusion category, as well as by regularly scheduled follow-up examinations by a trained trachoma grader. Should the antibiotic be ineffective to an individual, the study medication will

be discontinued for them. In the event of any adverse effects, appropriate medical care will be provided by the local health center.

14.4 Inclusion of Pregnant Women and Children

All participants, regardless of gender, will be accepted. We will obtain informed consent from all study participants prior to entering the study. Pregnant women will be excluded from receiving oral azithromycin, and will be offered topical tetracycline eye ointment in its place.

14.5 Compensation to Participants

There is no cost to the participant and there is no reimbursement for overall participation in this study. Each participant will receive free ophthalmic examinations during the course of the study.

Chapter 15

Data and Safety Monitoring

This chapter will define the primary responsibilities of the DSMC, its relationship with other trial components, its membership, and the purpose and timing of its meetings. This chapter will also provide the procedures for ensuring confidentiality and communication, statistical monitoring guidelines to be implemented by the DSMC, and an outline of the content of the reports that will be provided to the DSMC.

15.1 Primary Responsibilities of the DSMC

The DSMC will be responsible for safeguarding the interests of trial participants, assessing the safety and efficacy of the interventions during the trial, and monitoring the overall conduct of the trial. The DSMC will provide recommendations about stopping or continuing the trial. To contribute to the integrity of the trial, the DSMC may also formulate recommendations relating to the selection, recruitment, retention of participants, to protocol-specified regimens, and the procedures for data management and quality control.

A chief responsibility of the DSMC will be to review data on ocular chlamydia prevalence and make recommendations regarding the need for mass azithromycin treatments. The DSMC will make a decision about thresholds for initiating treatment at each meeting. For WUHA I, the DSMC purposefully did not set a threshold to trigger re-treatment, but rather a threshold triggering a discussion about whether antibiotic treatment was needed. This threshold was 20% prevalence of ocular chlamydia among the 0-5 year-old age group. It is anticipated that a similar threshold will be used for WUHA II, although the DSMC will discuss this issue at each in-person or telephone meeting.

The DSMC will be advisory to the trial leadership group, hereafter referred to as the Executive Committee (EC). The EC will be responsible for promptly reviewing the DSMC recommendations and determining, whether to continue or terminate the trial, and to determine whether amendments to the protocol are required. If needed, the DSMC may seek the advice of a content expert outside of the committee.

15.2 DSMC Membership

The DSMC is an independent multidisciplinary group consisting of epidemiologists, biostatisticians, bioethicists, and clinicians that collectively has experience in the management of infectious diseases and in the conduct and monitoring of randomized clinical trials including sub-Saharan Africa.

15.3 Conflicts of Interest

The DSMC membership has been restricted to individuals free of apparent conflicts of interest. The source of these conflicts may be financial, scientific, or regulatory. Thus, neither study investigators nor individuals employed by the sponsor, nor individuals who might have regulatory responsibilities for the trial products, are members of the DSMC.

The DSMC members will disclose to fellow members any consulting agreements or financial interests they have with the sponsor of the trial, with the contract research organizations (CRO) , or with other sponsors having products that are being evaluated or that are competitive with those in the trial. The DSMC will be responsible for deciding whether these consulting agreements or financial interests materially impact their objectivity.

The DSMC members will be responsible for advising fellow members of any changes in any of the membership requirements that occur during the course of the trial. It may be appropriate for DSMC members who develop significant conflicts of interest resign from the DSMC.

DSMC membership is to be for the full duration of the trial. If any members leave the DSMC, the EC, in consultation with the DSMC, will promptly appoint a replacement.

15.4 Timing and Purpose of the DSMC Meetings

15.4.1 Organizational Meeting

The initial meeting of the DSMC was held 16 September 2014. The committee provided an advisory review of scientific and ethical issues relating to study design and discussed the standard operating procedures, as well as the format and content of the reports that will be used to present trial results.

The Organizational Meeting was attended by all DSMC members, lead trial investigators, and the trial biostatistician. The DSMC reviewed drafts of the trial protocol, the Statistical Analysis Plan, and this DSMC chapter. At subsequent meetings, committee members will receive data reports and an updated MOP and SAP.

15.4.2 Future meetings

Future DSMC meetings will be held on a yearly basis, with an option for more frequent phone meetings or email updates at the discretion of the DSMC and/or principal investigator.

15.5 Procedures to Ensure Confidentiality and Proper Communication

To enhance the integrity and credibility of the trial, procedures will be implemented to ensure the DSMC has access to all emerging information from the trial regarding comparative results of efficacy and safety, aggregated by treatment arm.

15.6 Closed Sessions

Sessions involving only DSMC members and, where appropriate, those unmasked trial investigators (on the Data Coordinating Committee) who generate the Closed Reports (called Closed Sessions) will be held to allow discussion of confidential data from the trial, including information about the relative efficacy and safety of interventions.

At a final Closed Session, the DSMC will develop a consensus on its list of recommendations, including that relating to whether the trial should continue.

15.7 Open Sessions

In order for the DSMC to have access to information provided, by study investigators, or members of regulatory authorities, a joint session between these individuals and DSMC members will be held between the Closed Sessions.

15.8 Progress Reports

For each DSMC meeting, a report will be provided. The report will include data on recruitment and baseline characteristics, exam coverage, exam results, pooled data on eligibility violations, adverse events, and completeness of follow-up and compliance. The data analyst will prepare this report.

The report should provide information that is accurate, with follow-up that is complete to within 1-2 months of the date of the DSMC meeting. The Reports should be provided to DSMC members approximately three days prior to the date of the meeting.

15.9 Minutes of the DSMC Meeting

The research team will prepare minutes for the open portion of the meeting, including the DSMC's recommendations.

15.10 Recommendations to the Executive Committee (EC)

At each meeting of the DSMC during the trial, the committee will make a recommendation to the Executive Committee to continue or terminate the trial. This recommendation will be based primarily on safety and efficacy considerations and will be guided by statistical monitoring guidelines defined in this chapter.

Recommendations to amend the protocol or conduct of the study made by the DSMC will be considered and accepted or rejected by the EC. The EC will be responsible for deciding whether to continue or to stop the trial based on the DSMC recommendations.

The DSMC will be notified of all changes to the protocol or to study conduct. The DSMC concurrence will be sought on all substantive recommendations or changes to the protocol or study conduct prior to implementation.

The EC may communicate information in the Open Report to the sponsor and may inform them of the DSMC recommended alterations to study conduct or early trial termination in instances in which the EC has reached a final decision agreeing with the recommendation. The EC will maintain confidentiality of all information it receives other than that contained in the Open Reports until after the trial is completed or until a decision for early termination has been made.

Bibliography

[1] Anthony W Solomon, Martin J Holland, Neal D E Alexander, Patrick A Massae, Aura Aguirre, Angels Natividad-Sancho, Sandra Molina, Salesia Safari, John F Shao, Paul Courtright, Rosanna W Peeling, Sheila K West, Robin L Bailey, Allen Foster, and David C W Mabey. Mass Treatment with Single-Dose Azithromycin for Trachoma. *New Engl. J. Medicine*, 351(19):1962–1971, 2004.

[2] Emily S West, Beatriz Munoz, Harran Mkocha, Martin J Holland, Aura Aguirre, Anthony W Solomon, Robin Bailey, Allen Foster, David Mabey, and Sheila K West. Mass treatment and the effect on the load of Chlamydia trachomatis infection in a trachoma-hyperendemic community. *Investig. ophthalmology & visual science*, 46(1):83–87, 2005.

[3] B Thylefors, C R Dawson, B R Jones, S K West, and H R Taylor. A simple system for the assessment of trachoma and its complications. *Bull. World Heal. Organ.*, 65(4):477–483, 1987.

[4] Matthew J Burton, Martin J Holland, Pateh Makalo, Esther A N Aryee, Neal D E Alexander, Ansumana Sillah, Hannah Faal, Sheila K West, Allen Foster, Gordon J Johnson, David C W Mabey, and Robin L Bailey. Re-emergence of Chlamydia trachomatis infection after mass antibiotic treatment of a trachoma-endemic Gambian community: a longitudinal study. *Lancet (London, England)*, 365(9467):1321–1328, 4 2005.

[5] Sheila K West, Beatriz Munoz, Harran Mkocha, Martin J Holland, Aura Aguirre, Anthony W Solomon, Allen Foster, Robin L Bailey, and David C W Mabey. Infection with Chlamydia trachomatis after mass treatment of a trachoma hyperendemic community in Tanzania: A longitudinal study. *Lancet*, 366(9493):1296–1300, 2005.

[6] A H Skalet, V Cevallos, B Ayele, T Gebre, Z Zhou, J H Jorgensen, M Zerihun, D Habte, Y Assefa, P M Emerson, B D Gaynor, T C Porco, T M Lietman, and J D Keenan. Antibiotic selection pressure and macrolide resistance in Nasopharyngeal Streptococcus pneumoniae: A cluster-randomized clinical trial. *PLoS Medicine*, 7(12), 2010.

[7] S Haug, T Lakew, G Habtemariam, W Alemayehu, V Cevallos, Z Zhou, J House, K Ray, T Porco, T Rutar, J Keenan, T M Lietman, and B D Gaynor. The decline of pneumococcal resistance after cessation of mass antibiotic distributions for trachoma. *Clin. Infect. Dis.*, 51(5), 2010.

[8] B Ayele, A Aemere, T Gebre, Z Tadesse, N E Stoller, C W See, S N Yu, B D Gaynor, C E McCulloch, T C Porco, P M Emerson, T M Lietman, and J D Keenan. Reliability of measurements performed by community-drawn anthropometrists from rural Ethiopia. *PLoS ONE*, 7(1), 2012.

[9] D Pascolini and S P Mariotti. Global estimates of visual impairment: 2010. *Br. J. Ophthalmol.*, 96(5):614–618, 2012.

[10] Yemane Berhane, Alemayehu Worku, Abebe Bejiga, Liknaw Adamu, Wondu Alemayehu, Amir Bedri, Paul Courtright, Jamie Tallant, Rachel Carter Seligson, Vivian Singletary, Anyess Travers, West Gojjam, Survey Report, S P Mariotti, D Pascolini, J Rose-Nussbaumer, O F The, W H O Alliance, T H E Global, Elimination Of, Jeremiah Ngondi, Teshome Gebre, Estifanos B Shargie, Patricia M Graves, Yeshewamebrat Ejigsemahu, Tesfaye Teferi, Asrat Genet, Aryc W Mosher, Tekola Endeshaw, Mulat Zerihun, Ayenew Messele, Frank O Richards, Paul M Emerson, B Thylefors, C R Dawson, B R Jones, S K West, H R Taylor, More Neglected Tropical, W H O Alliance, Global Elimination, Blinding Trachoma, and Alliance Oms. Trachoma: global magnitude of a preventable cause of blindness. *The Br. J. Ophthalmol.*, 93(4):563–568, 3 2009.

[11] National Eye Institute. Vision Research: Needs, Gaps, and Opportunities, 2012.

[12] Silvio P Mariotti, Ramachandra Pararajasegaram, and Serge Resnikoff. Trachoma: looking forward to Global Elimination of Trachoma by 2020 (GET 2020). *The Am. journal tropical medicine hygiene*, 69(5 Suppl):33–35, 11 2003.

[13] World Health Organization. Meeting of the International Task Force for Disease Eradication — October 2010 = Réunion du Groupe spécial international pour l'éradication des maladies — octobre 2010, 2011.

[14] J D Keenan, P J Hotez, A Amza, N E Stoller, B D Gaynor, T C Porco, and T M Lietman. Elimination and Eradication of Neglected Tropical Diseases with Mass Drug Administrations: A Survey of Experts. *PLoS Neglected Trop. Dis.*, 7(12), 2013.

[15] Julius Schachter, Sheila K West, David Mabey, Chandler R Dawson, Linda Bobo, Robin Bailey, Susan Vitale, Thomas C Quinn, Ahmed Sheta, Sunny Sallam, Harran Mkocha, Denise Mabey, and Hannah Faal. Azithromycin in control of trachoma. *Lancet*, 354(9179):630–635, 3 1999.

[16] R L Bailey, P Arullendran, H C Whittle, and D C Mabey. Randomised controlled trial of single-dose azithromycin in treatment of trachoma. *Lancet (London, England)*, 342(8869):453–456, 3 1993.

[17] Jaya D Chidambaram, Wondu Alemayehu, Muluken Melese, Takele Lakew, Elizabeth Yi, Jenafir House, Vicky Cevallos, Zhaoxia X Zhou, Kathryn Maxey, David C Lee, Brett L Shapiro, Muthiah Srinivasan, Travis Porco, John P Whitcher, Bruce D Gaynor, and Thomas M Lietman. Effect of a single mass antibiotic distribution on the prevalence of infectious trachoma. *JAMA*, 295(10):1142–1146, 3 2006.

[18] Jenafir I House, Berhan Ayele, Travis C Porco, Zhaoxia Zhou, Kevin C Hong, Teshome Gebre, Kathryn J Ray, Jeremy D Keenan, Nicole E Stoller, John P Whitcher, Bruce D Gaynor, Paul M Emerson, and Thomas M Lietman. Assessment of herd protection against trachoma due to repeated mass antibiotic distributions: a cluster-randomised trial. *The Lancet*, 373(9669):1111–1118, 2009.

[19] Berna Atik, Ton Ton Kim Thanh, Vu Quoc Luong, Stephane Lagree, and Deborah Dean. Impact of annual targeted treatment on infectious trachoma and susceptibility to reinfection. *J. Am. Med. Assoc.*, 2006.

[20] Amza Abdou, Beatriz E. Munoz, Baido Nassirou, Boubacar Kadri, Fati Moussa, Ibrahim Baarè, Joseph Riverson, Emmanuel Opong, and Sheila K. West. How much is not enough? A community randomized trial of a Water and Health Education programme for Trachoma and Ocular C. trachomatis infection in Niger. *Trop. Medicine Int. Heal.*, 2010.

[21] J D Keenan, C W See, J Moncada, B Ayele, T Gebre, N E Stoller, C E McCulloch, T C Porco, B D Gaynor, P M Emerson, J Schachter, and T M Lietman. Diagnostic characteristics of tests for ocular chlamydia after mass azithromycin distributions. *Investig. Ophthalmol. Vis. Sci.*, 53(1), 2012.

[22] K G Roper and H R Taylor. Comparison of clinical and photographic assessment of trachoma. *The Br. journal ophthalmology*, 93(6):811–814, 3 2009.

[23] Mansur Rabiu, Mahmoud B Alhassan, Henry O D Ejere, and Jennifer R Evans. Environmental sanitary interventions for preventing active trachoma. *Cochrane Database Syst. Rev.*, (2):CD004003, 3 2012.

[24] Mansur Rabiu, Nasiru Muhammed, and Sunday Isiyaku. Challenges of trachoma control: An assessment of the situation in Northern Nigeria. *Middle East Afr. J. Ophthalmol.*, 2011.

[25] Sanitation Sector Status and Gap Analysis: Ethiopia, 2009.

[26] N Zerihun. Trachoma in Jimma zone, south western Ethiopia. *Trop. medicine & international health : TM & IH*, 2(12):1115–1121, 12 1997.

[27] T Gebre, B Ayele, M Zerihun, A Genet, N E Stoller, Z Zhou, J I House, S N Yu, K J Ray, P M Emerson, J D Keenan, T C Porco, T M Lietman, and B D Gaynor. Comparison of annual versus twice-yearly mass azithromycin treatment for hyperendemic trachoma in Ethiopia: A cluster-randomised trial. *The Lancet*, 379(9811), 2012.

[28] Muluken Melese, Wondu Alemayehu, Takele Lakew, Elizabeth Yi, Jenafir House, Jaya D Chidambaram, Zhaoxia Zhou, Vicky Cevallos, Kathryn Ray, Kevin Cyrus Hong, Travis C Porco, Isabella Phan, Ali Zaidi, Bruce D Gaynor, John P Whitcher, and Thomas M Lietman. Comparison of annual and biannual mass antibiotic administration for elimination of infectious trachoma. *JAMA*, 299(7):778–784, 2008.

[29] Juan D Gomez and Afamia C Nakat. Community Participation in Water and Sanitation. *Water Int.*, 27(3):343–353, 9 2002.

[30] B P Loevinsohn. Health education interventions in developing countries: a methodological review of published articles. *Int. journal epidemiology*, 19(4):788–794, 12 1990.

[31] N E Stoller, T Gebre, B Ayele, M Zerihun, Y Assefa, D Habte, Z Zhou, T C Porco, J D Keenan, J I House, B D Gaynor, T M Lietman, and P M Emerson. Efficacy of latrine promotion on emergence of infection with ocular Chlamydia trachomatis after mass antibiotic treatment: A cluster-randomized trial. *Int. Heal.*, 3(2), 2011.

[32] Maggie A Montgomery, Mayur M Desai, and Menachem Elimelech. Assessment of latrine use and quality and association with risk of trachoma in rural Tanzania. *Transactions Royal Soc. Trop. Medicine Hyg.*, 104(4):283–289, 4 2010.

[33] Valerie Curtis, Bernadette Kanki, Simon Cousens, Ibrahim Diallo, Alphonse Kpozehouen, Morike Sangare, and Michel Nikiema. Evidence of behaviour change following a hygiene promotion programme in Burkina Faso. *Bull. World Heal. Organ.*, 79:518–527, 2001.

[34] Juliet Waterkeyn and Sandy Cairncross. Creating demand for sanitation and hygiene through Community Health Clubs: a cost-effective intervention in two districts in Zimbabwe. *Soc. science & medicine (1982)*, 61(9):1958–1970, 11 2005.

[35] Adam Biran, Anara Tabyshalieva, and Zumrat Salmorbekova. Formative research for hygiene promotion in Kyrgyzstan. *Heal. policy planning*, 20(4):213–221, 7 2005.

[36] Thomas Clasen, Douglas Fabini, Sophie Boisson, Jay Taneja, Joshua Song, Elisabeth Aichinger, Anthony Bui, Sean Dadashi, Wolf-Peter Schmidt, Zachary Burt, and Kara L Nelson. Making sanitation count: developing and testing a device for assessing latrine use in low-income settings. *Environ. science & technology*, 46(6):3295–3303, 3 2012.

[37] Stephen P Luby, Mubina Agboatwalla, John Painter, Arshad Altaf, Ward L Billhimer, and Robert M Hoekstra. Effect of intensive handwashing promotion on childhood diarrhea in high-risk communities in Pakistan: a randomized controlled trial. *JAMA*, 291(21):2547–2554, 6 2004.

[38] Pavani K Ram, Amal K Halder, Stewart P Granger, Therese Jones, Peter Hall, David Hitchcock, Richard Wright, Benjamin Nygren, M Sirajul Islam, John W Molyneaux, and Stephen P Luby. Is structured observation a valid technique to measure handwashing behavior? Use of acceleration sensors embedded in soap to assess reactivity to structured observation. *The Am. journal tropical medicine hygiene*, 83(5):1070–1076, 11 2010.

[39] Philip C Hill, Abiodun Akisanya, Kawsu Sankareh, Yin Bun Cheung, Mark Saaka, George Lahai, Brian M Greenwood, and Richard A Adegbola. Nasopharyngeal carriage of Streptococcus pneumoniae in Gambian villagers. *Clin. infectious diseases : an official publication Infect. Dis. Soc. Am.*, 43(6):673–679, 9 2006.

[40] A Amza, B Kadri, B Nassirou, N E Stoller, S N Yu, Z Zhou, S K West, D C W Mabey, R L Bailey, J D Keenan, T C Porco, T M Lietman, and B D Gaynor. A cluster-randomized controlled trial evaluating the effects of mass azithromycin treatment on growth and nutrition in Niger. *Am. J. Trop. Medicine Hyg.*, 88(1), 2013.

[41] Alain B Labrique, Parul Christian, Rolf D W Klemm, Mahbubur Rashid, Abu Ahmed Shamim, Allan Massie, Kerry Schulze, Andre Hackman, and Keith P West Jr. A cluster-randomized, placebo-controlled, maternal vitamin A or beta-carotene supplementation trial in Bangladesh: design and methods. *Trials*, 12:102, 4 2011.

[42] Erica Brook Goodhew, Sheri Maria G Morgan, Andrew J Switzer, Beatriz Munoz, Laura Dize, Charlotte Gaydos, Harran Mkocha, Sheila K West, Ryan E Wiegand, Patrick J Lammie, and Diana L Martin. Longitudinal analysis of antibody responses to trachoma antigens before and after mass drug administration. *BMC infectious diseases*, 14:216, 4 2014.

[43] Diana L Martin, Ryan Wiegand, Brook Goodhew, Patrick Lammie, Carolyn M Black, Sheila West, Charlotte A Gaydos, Laura Dize, Harran Mkocha, Mabula Kasubi, and Manoj Gambhir. Serological Measures of Trachoma Transmission Intensity. *Sci. reports*, 5:18532, 12 2015.

[44] B P Pant, R C Bhatta, J S P Chaudhary, S Awasthi, S Mishra, S Sharma, P A Cuddapah, S E Gwyn, N E Stoller, D L Martin, J D Keenan, T M Lietman, and B D Gaynor. Control of Trachoma from Achham District, Nepal: A Cross-Sectional Study from the Nepal National Trachoma Program. *PLoS Neglected Trop. Dis.*, 10(2), 2016.

[45] S K West and H R Taylor. Reliability of photographs for grading trachoma in field studies. *The Br. journal ophthalmology*, 74(1):12–13, 1 1990.

[46] Anthony W Solomon, Richard J C Bowman, David Yorston, Patrick A Massae, Salesia Safari, Brian Savage, Neal D E Alexander, Allen Foster, and David C W Mabey. Operational evaluation of the use of photographs for grading active trachoma. *The Am. journal tropical medicine hygiene*, 74(3):505–508, 3 2006.

[47] Jens Kjølseth Møller, Lisbeth Nørum Pedersen, and Kenneth Persson. Comparison of the Abbott RealTime CT New Formulation Assay with Two Other Commercial Assays for Detection of Wild-Type and New Variant Strains of Chlamydia trachomatis. *J. Clin. Microbiol.*, 48(2):440 LP – 443, 2 2010.

[48] Annie Cheng, Qinfang Qian, and James E Kirby. Evaluation of the Abbott RealTime CT/NG assay in comparison to the Roche Cobas Amplicor CT/NG assay. *J. clinical microbiology*, 49(4):1294–1300, 3 2011.

[49] Jens Kjølseth Møller, Lisbeth Nørum Pedersen, and Kenneth Persson. Comparison of the Abbott RealTime CT new formulation assay with two other commercial assays for detection of wild-type and new variant strains of Chlamydia trachomatis. *J. clinical microbiology*, 48(2):440–443, 3 2010.

[50] S A Morré, R van Dijk, C J Meijer, A J van den Brule, S K Kjaer, and C Munk. Pooling cervical swabs for detection of Chlamydia trachomatis by PCR: sensitivity, dilution, inhibition, and cost-saving aspects. *J. clinical microbiology*, 39(6):2375–2376, 3 2001.

[51] Jonathan Diamant, Roger Benis, Julius Schachter, Jeanne Moncada, Frankye Pang, Hem C Jha, Ramesh C Bhatta, J S P Chaudhary, Travis Porco, and Tom Lietman. Pooling of Chlamydia laboratory tests to determine the prevalence of ocular Chlamydia trachomatis infection. *Ophthalmic Epidemiol.*, 8(2-3):109–117, 1 2001.

[52] Hendrik J M de Jonge, Rudolf S N Fehrmann, Eveline S J M de Bont, Robert M W Hofstra, Frans Gerbens, Willem A Kamps, Elisabeth G E de Vries, Ate G J van der Zee, Gerard J te Meerman, and Arja ter Elst. Evidence based selection of housekeeping genes. *PloS one*, 2(9):e898, 9 2007.

[53] Eva V Basilion, Peter M Kilima, and Jeffrey W Mecaskey. Simplification and improvement of height-based azithromycin treatment for paediatric trachoma. *Transactions Royal Soc. Trop. Medicine Hyg.*, 99(1):6–12, 3 2005.

[54] Milton C Weinstein, Bernie O'Brien, John Hornberger, Joseph Jackson, Magnus Johannesson, Chris McCabe, and Bryan R Luce. Principles of good practice for decision analytic modeling in health-care evaluation: report of the ISPOR Task Force on Good Research Practices–Modeling Studies. *Value health : journal Int. Soc. for Pharmacoeconomics Outcomes Res.*, 6(1):9–17, 2003.

[55] Scott Ramsey, Richard Willke, Andrew Briggs, Ruth Brown, Martin Buxton, Anita Chawla, John Cook, Henry Glick, Bengt Liljas, Diana Petitti, and Shelby Reed. Good research practices for cost-effectiveness analysis alongside clinical trials: the ISPOR RCT-CEA Task Force report. *Value health : journal Int. Soc. for Pharmacoeconomics Outcomes Res.*, 8(5):521–533, 2005.

Appendix A

Revision History

11 March 2020

- Converted MOP from word doc to Overleaf doc
- Re-organized sectioning of MOP chapters
- Added more specific information on data collection and management plan

4 March 2022

- Specified if a round of MDA takes place within the 3 months prior to a round of examinations, the results from that examination will not be included in the final analysis, since the mass drug administration would be expected to greatly reduce infection in both treatment arms, potentially masking the effect of the hygiene intervention.

Sanitation, Water, and Instruction in Face-washing for Trachoma I

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CONTENTS

1. INTRODUCTION

Trachoma is one of the few realistic candidate diseases that could be eradicated in the next two decades. The chances of eradication stem partly from the use of mass azithromycin distributions, which have had enormous success in reducing the prevalence of the ocular strains of chlamydia that cause the disease. However, recent evidence suggests that antibiotics alone may be insufficient for eradication. There are at least 3 limitations of mass antibiotic distributions for trachoma. First, the efficacy of azithromycin in clearing infection may not be as high as previously thought. In severely affected areas such as Ethiopia, even 7 mass antibiotic distributions fail to predictably eliminate chlamydia—and infection rapidly returns after treatments are discontinued. Second, mass antibiotic distributions select for resistance. While no resistance has yet been observed in chlamydia, mass azithromycin clearly selects for resistance in nasopharyngeal pneumococcus. And third, adverse events are possible. Non-serious side effects are not uncommon, and serious side effects are possible, with a recent study showing an association between azithromycin and sudden death in hospitalized patients. The inability of even frequent mass azithromycin distributions to bring about elimination in areas with highly prevalent trachoma suggests that other, non-antibiotic measures may be needed.

Improvements in water and sanitation are thought to change the transmission dynamics of ocular chlamydia, and may be necessary to eliminate trachoma in the most hyperendemic locations. Historically, trachoma disappeared in many areas before widespread use of antibiotics, often concomitant with improvements in environmental public health measures. Lack of water and sanitation, along with poor facial hygiene, have long been known to be associated with trachoma, and the World Health Organization (WHO) recommends facial hygiene and environmental improvements as important components of their *SAFE* strategy for trachoma (Surgery, Antibiotics, Facial hygiene, and Environmental improvements).

Water and sanitation interventions have yet to be proven effective for reducing ocular chlamydia. The randomized clinical trials of water, sanitation, and hygiene (WASH) interventions that have been conducted for trachoma have typically been underpowered. Those that monitored clinical disease did so with a grading scale that may not be sensitive enough for research purposes. The few studies that monitored ocular chlamydial infection did so with an earlier generation, less sensitive PCR test (Roche Amplicor) that may have misclassified some infections. None of the prior trials monitored infectious load of chlamydia, which is thought to be important for transmission. Furthermore, few of the trials have implemented multiple hygiene interventions together. It is possible that the effectiveness of each of the WASH components depends on the others. Finally, there is general consensus that non-antibiotic measures may take longer to work than the 1-2-year duration of most previous trials. *We believe that the lack of evidence does not indicate that these measures are ineffective—simply that they have not been adequately assessed.*

Our long-term goal is to eliminate trachoma even in the most hyperendemic communities. In order to advance this goal, we will conduct a cluster-randomized clinical trial to determine the role of a comprehensive package of sanitation measures for the elimination of trachoma. We will monitor clinical disease with photography, and monitor infection with a newer chlamydial PCR test (Abbott *m*2000) that is more sensitive than earlier generation tests, and provides quantification. We will monitor other potential health benefits of a WASH intervention and test its overall cost effectiveness. We will also assess a competing strategy for minimizing antibiotic use: that of targeted azithromycin treatments to children testing positive for ocular chlamydia. Our central hypothesis is that instituting an integrated WASH package including water points, latrines, and facial hygiene promotion will reduce both the prevalence and the severity of both clinical disease and infection. If these measures prove effective and analyses demonstrate a favorable cost-effectiveness profile, the implementation of these interventions would increase dramatically.

1.1 Specific Aims of the Study

Specific Aim 1: To determine the efficacy of non-antibiotic measures for trachoma control.

- *Hypothesis 1:* The prevalence of ocular chlamydia will be lower in clusters randomized to a WASH package compared to clusters not receiving this intervention.

Specific Aim 2: To determine the efficacy of targeting antibiotics to infected children for trachoma control.

- *Hypothesis 2A:* The incidence of ocular chlamydia in uninfected pre-school children will be lower in clusters where infected pre-school children are periodically treated with azithromycin than in clusters not treated with azithromycin.
- *Hypothesis 2B:* The prevalence of ocular chlamydia in clusters randomized to periodic targeted azithromycin treatments will be non-inferior to those treated with a single mass azithromycin treatment.

Specific Aim 3: To model the long-term cost-effectiveness of competing strategies for trachoma control after completion of several rounds of mass azithromycin distributions.

- *Hypothesis 3:* We will assess the incremental cost effectiveness of an integrated WASH package versus a targeted antibiotic strategy versus no specific intervention over a 10-year time horizon, and anticipate that the incremental cost effectiveness will favor the WASH package over the long-term.

This research will provide the strongest type of evidence—that from a randomized clinical trial—to guide treatment decisions for trachoma programs. With less than 10 years until the WHO's 2020 target date for trachoma programs, this research will provide an assessment of the current SAFE strategy and equip trachoma programs to spend their limited resources on interventions most likely to result in elimination. The research will have applicability even if trachoma elimination is achieved by 2020, since it will determine whether WASH interventions are able to maintain trachoma elimination once mass antibiotics have stopped

1.2 Study Outcomes

1.2.1 ***Primary Outcomes:***

The primary outcome is ocular chlamydia as assessed by PCR, measured in a population-based age-stratified sample of the entire community. We age-stratify since the risk of ocular chlamydial infection is strongly associated with age (i.e., infection much more common in younger children), but trachoma elimination will require elimination of infection from the entire community, so infection data in older age groups is of interest.[1-4]

1.2.2 ***Secondary Outcomes:***

The secondary outcome of most interest is the infectious load of chlamydia among 0-5-year-old children infected with ocular chlamydia. Individuals with high chlamydial loads are thought to be much more likely to transmit infection.[5] Therefore, it is conceivable that reduction of chlamydial load is more important in the short term than reducing the prevalence of chlamydia, since individuals with low loads will be less likely to propagate the infection. However, because this outcome has never been assessed as part of a randomized clinical trial, we have designated it as a secondary outcome. Our other secondary outcome of highest interest is improvement in conjunctival inflammation as assessed from serial conjunctival photographs, measured in a longitudinal cohort of children aged 0-5 years at baseline. We will collect data on several other secondary outcomes in children aged 0-5 years, including dried blood spots for serological assessment of ocular chlamydia and other diseases of interest, nasopharyngeal pneumococcal macrolide resistance, anthropometry (height and weight), the presence of a clean face on photography, stool samples for the presence of *Necator americanus*, *Ancylostoma duodenale*, *Trichuris trichiura*, *Ascaris lumbricoides*, and *Strongyloides stercoralis*, for the presence and density of soil-transmitted helminths. For WUHA, we will perform similar assessments of ocular chlamydia and clinical trachoma in a sample of children aged 6-9 years and a sample of individuals aged ≥ 10 years, and similar assessments of facial cleanliness in the 6-9-year-old age group. For TAITU, we will perform similar assessments of ocular chlamydia and clinical trachoma in a sample of children aged 8-12 years. We are monitoring for macrolide resistance in nasopharyngeal pneumococcus because azithromycin treatments may select for antibiotic resistance and because of the possibility that improved hygiene will have an added benefit of reducing the carriage of nasopharyngeal pneumococcus. We are monitoring anthropometry because of the possibility that improved hygiene will result in accelerated growth in pre-school children. Our group has measured both nasopharyngeal macrolide resistance and anthropometry in previous trials in Ethiopia.[6-8]

1.2.3 ***Research Design***

We will conduct a series of cluster-randomized clinical trials with long-term follow-up to determine whether adding an integrated WASH package to mass azithromycin treatment will be

effective for trachoma. We will conduct two complementary trials to take place in 80 clusters of households that have already received five or more rounds of mass azithromycin treatments. Specific Aim 1 describes the first trial, in which 40 clusters will be randomized either to an integrated WASH package (water points, latrine promotion, and hygiene promotion) or to no intervention until the conclusion of the study. The second trial, described in Specific Aim 2, studies a competing antibiotic minimization strategy in which 36 clusters will be randomized either to targeted azithromycin treatments of children infected with ocular chlamydia or to a single mass azithromycin distribution. The primary outcome for both trials will be ocular chlamydia infection, assessed in 0-5 year-old children at months 12, 24, and 36 for the first trial and assessed in both 0-5- and 8-12-year-old children at month 12 and 24 for the second trial. Important secondary outcomes will include the load of ocular chlamydia among infected children and the prevalence of clinically active trachoma, assessed at the same time points.

2. BACKGROUND AND RATIONALE

Trachoma is the most common infectious cause of blindness worldwide.[9] Caused by ocular strains of chlamydia, over 40 million people are currently affected with active trachoma—mostly in sub-Saharan Africa.[10] Ocular chlamydia infection causes conjunctival inflammation, with characteristic white follicles (germinal centers) present on the upper tarsal conjunctiva. Frequent bouts of infection and inflammation result in cicatricial changes to the conjunctiva, with subsequent inturning of the eyelids (entropion). With this this inturned eyelid position, eyelashes touch the globe (trichiasis), causing pain and creating the conditions for corneal abrasions, bacterial and fungal superinfections, corneal opacity, and blindness. The National Eye Institute's most recent 5-year plan encourages clinical trial interventions for blinding global eye diseases, and specifically urges research in connection with trachoma control programs to cost-effectively test optimal intervention strategies for global infectious causes of blindness.[11]

The World Health Organization has called for elimination of trachoma as a public health problem by the year 2020.[12] Through its Global Elimination of Trachoma by 2020 (GET 2020) initiative, the WHO has designated trachoma a priority eye disease, and has brought together academic institutions, nongovernmental organizations, and pharmaceutical companies to promote elimination of this blinding condition. Trachoma may even be a candidate for eradication. Many countries in Asia and the Middle East have had great success in reducing the prevalence of trachoma, and no longer require active trachoma control efforts.[13] If these experiences can be replicated in the heaviest affected areas of Africa, trachoma may be the first bacterial disease ever to be eradicated from the planet.

Although antibiotics have proven highly effective in reducing ocular chlamydia, many believe that trachoma elimination will require non-antibiotic measures. There are several reasons why antibiotics may not be the sole pathway to eliminate trachoma. First, they may not be effective enough: multiple rounds of antibiotic distributions have not been shown to eliminate ocular chlamydia in areas with moderate to high amounts of trachoma—even when treatments are given as frequently as every 3-6 months.[14-17] Second, they may cause adverse events. While mass antibiotic distributions have not been shown to induce chlamydial resistance, they do select for resistant strains of commensal bacteria such as nasopharyngeal *Streptococcus pneumoniae*.[6,18] Resistant strains have so far remained susceptible to penicillin, but clonal spread of commensal bacteria resistant to both macrolides and penicillins could limit the role of mass azithromycin distributions in the future. Although azithromycin generally has a favorable side effect profile, some believe that azithromycin may cause sudden death in rare instances.[19] Finally, they may not be necessary: trachoma was eliminated in the United States long before organized mass antibiotic efforts, and has even been dramatically reduced in some African countries—likely as a result of improvements in public health and sanitation.[20-22]

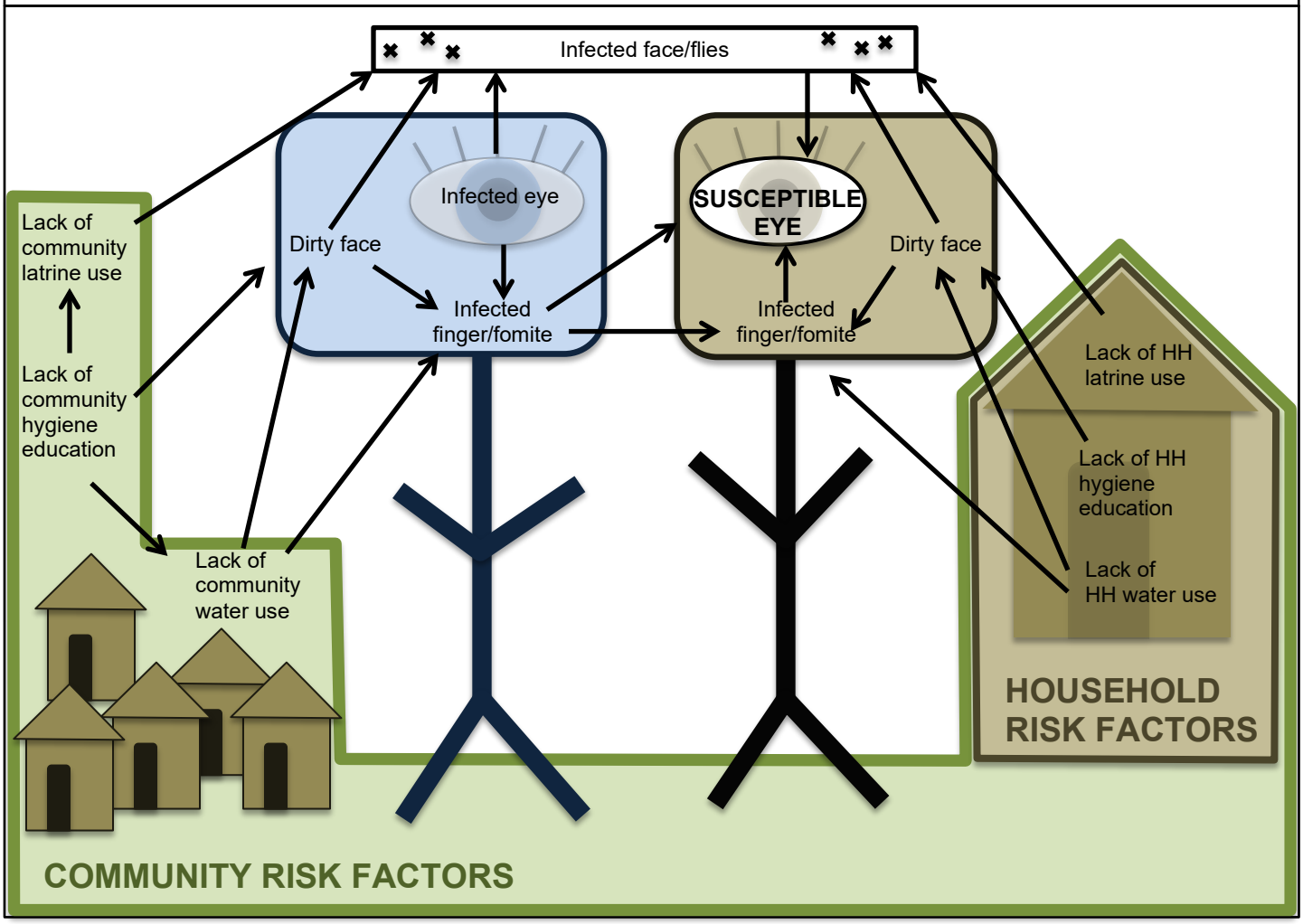
The World Health Organization recommends several non-antibiotic measures in its "SAFE" strategy for trachoma control. The components of the SAFE strategy include ***S***urgical correction

of trichiasis, ***A***ntibiotics to reduce the community load of ocular chlamydia, and ***F***acial hygiene and ***E***nvironmental improvements to reduce the transmission of ocular chlamydia. The rationale for the SAFE strategy is based on many years of observational studies on trachoma. Cross-sectional studies have found that clinically active trachoma and ocular chlamydial infection are associated with several indicators of poor hygiene, including dirty faces, face-seeking flies, long distance to water supply, and lack of household latrine.

There are only a handful of randomized trials testing a water, sanitation, or hygiene intervention for trachoma. Most of these trials have been unable to detect any effect of the tested intervention, though there is some evidence supporting hygiene education.[23,24] None of the trials has been able to detect a difference in ocular chlamydia due to the intervention. Although this may mean that water and sanitation interventions have no effect on ocular chlamydia, it is also possible that the limitations of these trials prevented a true effect from being found. For example, the trials were typically underpowered and had short follow-up. Most trials monitored only for clinically active trachoma, which is an unreliable indicator of ocular chlamydial infection.[25] Those trials that have monitored for ocular chlamydia have generally been underpowered.[26,27] None provided all 3 components (e.g., water, sanitation, and hygiene) simultaneously. The state of evidence regarding environmental modifications for trachoma control is perhaps best summarized by the 2012 Cochrane Review article on this subject, which concluded: "Generally there is a dearth of data to determine the effectiveness of all aspects of environmental sanitation in the control of trachoma."[28] Perhaps because of this lack of evidence, many trachoma programs focus their activities around the "S" (surgery) and "A" components (antibiotics), while neglecting the "F" (facial hygiene) and "E" (environmental improvements) components of the SAFE strategy.[29]

We expect to determine whether an integrated approach to improve access to water, sanitation and hygiene (WASH) technologies and related behaviors (i.e., the "F" and "E" components of SAFE) is effective in reducing the prevalence of ocular chlamydia. Although no single water, sanitation, or facial hygiene intervention has been shown to be effective for trachoma, it is possible that roll-out of the entire package of interventions will be effective—as has been voiced by several trachoma experts and endorsed by the WHO.[30,31] This trial will provide the first attempt to test this hypothesis, while also determining whether any benefits of the interventions are also cost-effective. If we find a reduction in ocular chlamydia with all interventions together, this would provide motivation to trachoma programs to include these non-antibiotic measures in their program activities. Further studies could be done to determine which of the "F" and "E" components are most important. If we fail to find a reduction in ocular chlamydia, this would trigger re-assessment of the ability of programs to alter transmission of ocular chlamydia through non-antibiotic measures, and would argue for new ways of implementing face hygiene promotion and environmental improvements. *The results of this study could be used immediately by trachoma programs when planning program activities, and therefore promise to have a major impact on the effort to eliminate blinding trachoma.*

Figure 1: Putative causal framework depicting relationships between hygiene factors and ocular chlamydia infection. In the diagram, the blue figure represents the infected individual, who can transmit ocular chlamydia through fingers, flies, and fomites. The brown figure represents the uninfected, susceptible individual. The susceptible individual's household risk factors are on the right, and the community risk factors are listed on the left. Lack of latrines promotes fly populations, which can transmit ocular chlamydia. Lack of water and hygiene awareness promotes dirty faces, which can be a reservoir for chlamydia and attract flies. Note that while household sanitation and hygiene play a role in the transmission of ocular chlamydia for household members, the community's participation in sanitation and hygiene is also important.



3. ORGANIZATION AND POLICIES

3.1 Study Organization

3.1.1 ***Study Partners***

The Proctor Foundation and Carter Center are the ideal partners to conduct the proposed research. They have worked together on 2 large NEI-funded cluster-randomized clinical trials in Ethiopia starting in 2006, and are currently collaborating on a Gates Foundation-funded trial of mass azithromycin in Niger. The research partners complement each other, with the Proctor Foundation providing expertise in study design and analysis, and the Carter Center implementing the field work in a thorough yet efficient manner. Matthew Freeman from Emory University will also be a crucial member of the research collaboration; Dr. Freeman is a WASH expert who has conducted cluster-randomized trials of WASH interventions in Africa and has experience with qualitative methods.

3.1.2 ***Executive Committee***

The Executive Committee will consist of Dr. Jeremy Keenan from the Proctor Foundation and Kelly Callahan from the Carter Center. This committee will act as the administrative and executive arm of the clinical trial and will meet in person twice per year to provide overall oversight for the study and make decisions on day-to-day operation issues as described in the following:

- Monitor study progress and data collection process
- Discuss any quality control issues that have arisen in the Trial Coordinating Center (TCC) and Data Coordinating Center (DCC)
- Evaluate and adopt changes in study procedures as necessary
- Communicate with and implement recommendations from the Data and Safety Monitoring Committee (DSMC)
- Make executive decisions on the allocation of resources
- Establish policies on publications and authorship
- Approve and oversee ancillary studies

3.1.3 ***Trial Coordinating Center (TCC)***

The TCC will be located at the Proctor Foundation. Dr. Jeremy Keenan will lead the center, which will also include Dr. Thomas Lietman (co-investigator), Dr. Travis Porco (biostatistician), Dionna Wittberg (Proctor program manager), Jason Melo (statistical analyst), and Vicky Cevallos (Proctor microbiologist). The role of the TCC will be to oversee and coordinate the overall implementation of the trial. Specifically, this means maintaining an up-to-date manual of procedures, obtaining ethical approvals from all involved parties (UCSF, Carter Center, and Ethiopia), leading development of study materials, helping with implementation of the study activities, conducting

training and certification of all study personnel, ensuring proper masking of outcome assessment, and monitoring adherence and adoption of the study intervention. The TCC will organize site visits at least twice per year before each monitoring visit to conduct training sessions with outcome assessors and monitor the quality of data collection. Either the principal investigator or the Proctor program manager will be present at all site visits. The Proctor program manager, with assistance from the Carter Center study coordinator will organize the training sessions and oversee the design and production of all educational messaging used in the hygiene promotion. The TCC will meet officially as a group at least 4 times per year. All members of the TCC are currently working on studies of trachoma in Ethiopia and Niger. The group has close working relationships with the Carter Center staff in Ethiopia, and experience with cluster-randomized trials in resource-poor settings.

3.1.4 ***Ethiopian Coordinating Center***

The Ethiopian Coordinating Center will be located at the Carter Center Ethiopia headquarters in Addis Ababa, Ethiopia. The center will be led by the study site principal investigator, Dr. Zerihun Tadesse, and also include the Carter study coordinator, Dr. Solomon Aragie. Dr. Zerihun will oversee the study activities that take place in Ethiopia, and will manage the day-to-day activities of the Carter study coordinator. He will also assist with obtaining ethical approval from appropriate federal, regional, and zonal agencies. He is supervised by Kelly Callahan from the Atlanta headquarters of the Carter Center. The Carter Center study coordinator (Dr. Solomon Aragie) will work with the local health officials to find workers for the census activities, nurses, data collectors, and lab staff to work as data collectors, a hygiene cluster coordinator, and health promotion workers (HPWs). He will liaise with Catholic Relief Services (the organization that will install the water points) and will also assist with synthesizing feedback from the formative research. He will assist with the WASH intervention development, and he will also be responsible for implementation of the WASH intervention. He will supervise all data collection activities and will be responsible for proper storage and transport of specimens. He will be responsible for dealing with the Amhara Public Health Institute to ensure the laboratory has adequate supplies and processes the specimens in a timely fashion. Finally, he will coordinate data entry of the costing forms, which will be done by experienced data entry staff at the Carter Center headquarters in Addis Ababa.

3.1.5 ***Data Coordinating Center (DCC)***

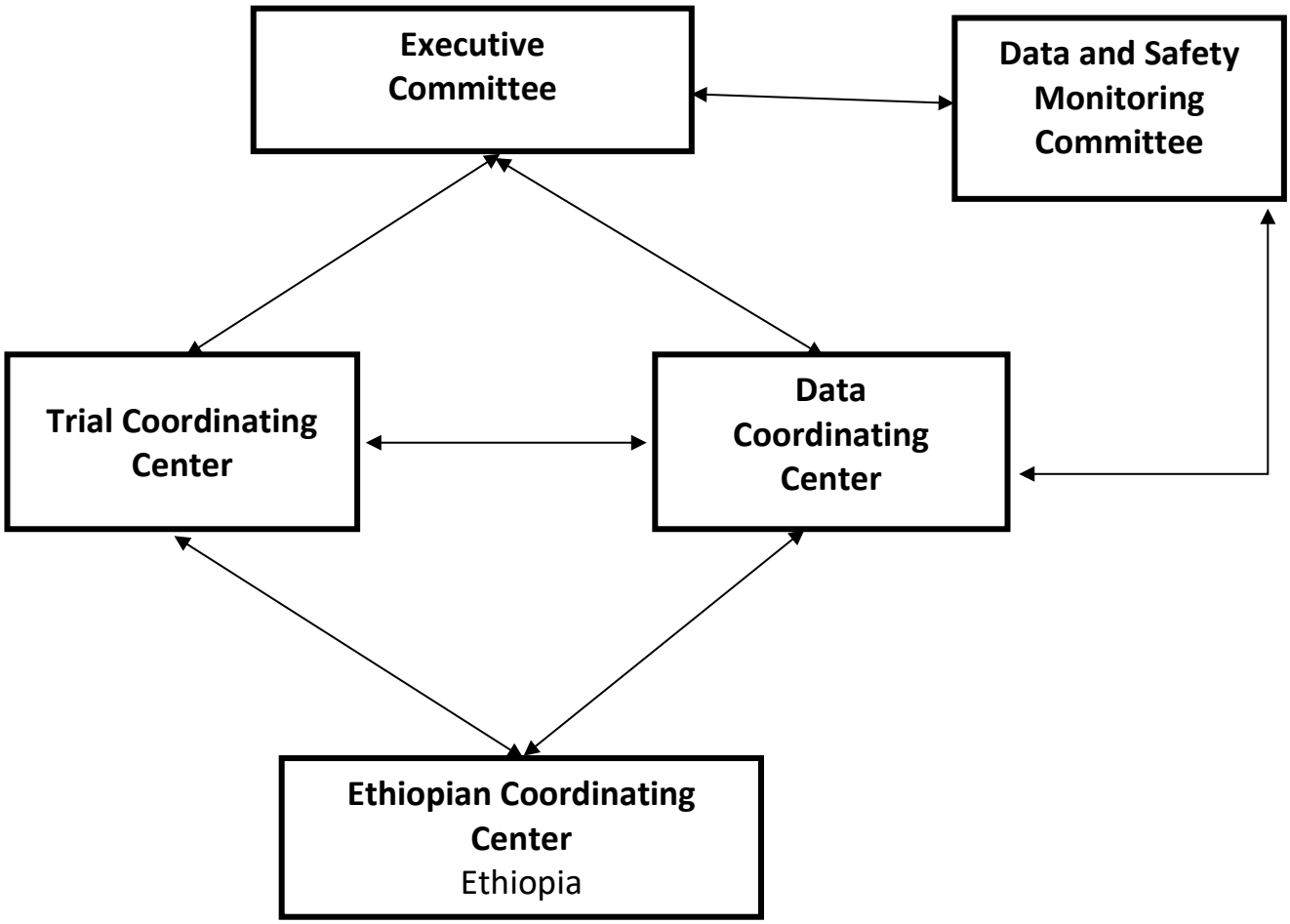
The DCC will be located at the Proctor Foundation, and led by Dr. Travis Porco. The Proctor Foundation has served as a DCC for several other trials, including cluster-randomized trials of trachoma in Ethiopia (TANA and TIRET continuation study, NEI U10 EY016214) and Niger (MORDOR; Gates OPP 1032340), and individual cluster-randomized trials of corneal ulcers in India (SCUT, NEI U10 EY015114; MUTT, NEI U10 EY018573). Dr. Porco, Dionna Wittberg (program manager), and Jason Melo (statistical analyst) will be responsible for data management, data quality control, event adjudication, and training and certification of data entry personnel. The DCC will be responsible for drafting the trial's statistical analysis plan, analyzing data according to the plan, and providing data requested for publications. The DCC will be responsible for

coordinating and supervising the activities of the Data and Safety Monitoring Committee, including preparing interim and final data reports. The DCC will also be responsible for coordinating the use of the electronic data capture system, including maintenance of the software application and data backup monitoring visits. The DCC will be in close contact with the Ethiopian study coordinator to help address any issues in Ethiopia.

3.1.6 ***Data and Safety Monitoring Committee (DSMC)***

The DSMC has been formed according to NIH guidelines and is comprised of independent experts in bioethics, biostatistics, epidemiology, ophthalmology, and international health. The DSMC will meet at least once per year. Ad hoc meetings may be convened as needed. The DSMC approved the trial protocol at the first meeting (16 September 2014). At annual meetings, the DSMC will review data on effectiveness outcomes. A chief responsibility will be to decide whether ocular chlamydia infection levels become high enough (around 20% in study clusters) to necessitate treatment with mass azithromycin. They will also monitor for unanticipated events.

Figure 2: SWIFT Study Organization Chart



3.2 Collaborating Institutions

3.2.1 ***Francis I. Proctor Foundation***

The Proctor Foundation is an organized research unit at the University of California, San Francisco. The Foundation has a 72 year history of research in ocular infectious and inflammatory diseases and runs one of the leading corneal fellowship training programs in the United States. Proctor Foundation Faculty has been involved in prevention of blindness research in developing countries since the foundation's inception. The impetus for establishing the foundation in 1947 was to eradicate trachoma in the American Southwest and in other parts of the world.

From this initial vision we have expanded our research efforts to include the other major causes of blindness worldwide, with a continuing emphasis on infectious and inflammatory eye diseases. The Proctor Foundation will be the main coordinating center for the study. Dr. Jeremy Keenan, Principal Investigator of the study at Proctor Foundation, will be assisted by numerous co-investigators, a program manager, a study coordinator, a microbiologist, a data analyst, laboratory PCR processing staff, and a biostatistician.

Our experience working in Ethiopia has been very successful to date – with the TEF (Trachoma Elimination and Follow-up) study in the Gurage region, TANA (Trachoma Amelioration in Northern Amhara) and the TIRET (Tripartite International Research for the Elimination of Trachoma) study in the Amhara region. We have partnered with ORBIS International and The Carter Center, respectively, to conduct/complete these large-scale projects.

3.2.2 ***The Carter Center – Ethiopia***

The Carter Center is guided by a fundamental commitment to human rights and the alleviation of human suffering; it seeks to prevent and resolve conflicts, enhance freedom and democracy and improve health. The trachoma control program does not just fight disease, it fights the conditions that perpetuate disease: poverty, poor sanitation, lack of knowledge, and hopelessness. Together, the Carter Center and its program partners are working to build a brighter future for those at risk for this devastating disease**.**

The focus of the country program has been on trachoma control, and delivery of quality eye care and blindness prevention programs to the majority of the population that live in rural areas (72%), largely unserved by trained eye care professionals.

Partnering with the Carter Center has yielded very positive results. The organization has an established infrastructure for large scale health promotion and improvement programs, as well as a strong working relationship with the Ethiopian Ministry of Health – both of which have contributed to the success of our field research.

3.2.3 Amhara Public Health Institute (formerly Bahir Dar Regional Health and Research Laboratory) – Ethiopia

The Amhara Public Health Institute in Bahir Dar, Ethiopia was established to be a center of excellence for the development of laboratory systems in the region. Construction of this CDC and USAID supported laboratory was completed in 2011. Conjunctival swabs will be processed with the Abbott RealTi*m*e assay for Chlamydia trachomatis, using the automated Abbott *m*2000 System, which is already in use at the laboratory. Amhara Public Health Institute will also process the swabs using media selective for Streptococcus pneumoniae, and then test for antibiotic resistance to erythromycin, penicillin, tetracycline, and clindamycin using the Kirby Bauer disc diffusion assay.

3.2.4 Emory University

The Rollins School of Public Health comprises six academic departments: behavioral sciences and health education, biostatistics, environmental and occupational health, epidemiology, health policy and management, global health, and hosts over 20 interdisciplinary centers. Its location in Atlanta provides ample interaction with other public health professionals interested in global health. Specifically, the school has close ties with the Carter Center and the Centers for Disease Control and Prevention, both of which are in Atlanta and contribute faculty to the school. Dr. Matthew Freeman has a dual appointment in the Department of Environmental Health and in the Hubert Department of Global Health.

3.2.5 Centers for Disease Control and Prevention

Dr. Diana Martin and her lab at the Centers for Disease Control and Prevention are developing new serological tests to monitor trachoma elimination as well as other diseases of interest including helminths, intestinal bacterial pathogens, and intestinal protozoal pathogens. Dr. Martin's lab will complete serological testing for trachoma and other diseases of interest using the dried blood spots collected throughout the study.

3.3 Duties & Responsibilities of Staff

3.3.1 Principal Investigator (Proctor Foundation)

- Develop study design, specific aims and outcome measures, with help of biostatistician, study coordinators, and partners
- Obtain grant funding with help of partners
- Ensure that staff follow through on protocol and properly execute all areas of research
- Ensure that all ethical approvals are maintained
- Oversee intervention implementation
- Write or add major contributions to all study-related publications
- Ensure proper masking procedure for staff involved in the study
- Supervise training and certification for all trachoma examiners

3.3.2 Program Manager (Proctor Foundation)

- Ensure the execution of the study per protocol

- Coordinate with the collaborating center, The Carter Center, Ethiopia (TCCE) and particularly with the TCCE Study Coordinator, in execution of the study
- Manage correspondence between all collaborating organizations and parties
- Maintain all clearances for the study, including IRB renewals, NRERC, the Ethiopian FDA (formerly FMHACA, the BUA, and DSMC-related approvals.
- Manage budgetary items including the NIH award and subcontracts
- In collaboration with TCCE Study Coordinator, prepare the census and examination application, electronic tools, and paper forms necessary for fieldwork (cluster registration lists, spot check REDCap, costing forms, mini-spot check household lists, and etc.)
- Train census and exam workers in the use of the electronic data capture system, with help of TCCE Study Coordinator
- Train both Proctor and Ethiopian health worker teams for each collection visit; direct team, with help of TCCE Study Coordinator, while in Ethiopia
- Train and certify Hygiene Cluster Coordinators and health promotion workers (HPWs) with help of TCCE Study Coordinator
- Direct development and implementation of WASH intervention
- Oversee data collection efforts and ensure data is analyzed in a timely manner
- Arrange logistics and itineraries for traveling team members in Ethiopia
- Purchase, maintain, and organize transport of all necessary study supplies to/within Ethiopia
- Maintain communication and partnership with Principal Investigator regarding all study activities and plans

*3.3.3 **Study Coordinator (The Carter Center, Ethiopia)***

- Prior to the start of the study, secure support from the Amhara Regional Health Bureau, and written approvals from the NRERC (Ministry of Science and Technology) and the Ethiopian FDA
- At baseline and throughout the study, obtain and maintain permission from zonal health leaders for the SWIFT study. Meet with and obtain permission/consent from all kebele and sub-kebele leaders.
- Train and supervise census and household survey team, including training in the electronic data capture system
- Supervise and train exam data collection teams (with emphasis on any new members) throughout each collection, especially after departure of Proctor study team
- Manage the data collected during the census and exams by ensuring that the app data is uploaded to Salesforce and that the exam photographs are saved on external hard drives
- Arrange printing of study materials including educational materials for households, hygiene workshops, schools, and advertising
- Train, certify, and work closely with the Hygiene Cluster Coordinators and the HPWs (with help of Proctor Study Coordinator)
- Train and supervise all albendazole and antibiotic MDA distribution teams
- Maintain all fieldwork documents and records
- Complete all collection and intervention reports
- Work with Data Analyst to maintain census and exam database

- Help develop and implement WASH interventions
- Oversee transport of study samples to Amhara Public Health Institute
- Assist Proctor Coordinators in coordinating logistics in Ethiopia
- Train and supervise data entry staff at Carter Center Headquarters in Addis Ababa
- Analyze and provide data when requested by co-investigators or staff from Proctor Foundation
- Appropriately back-up all data

*3.3.4 **Co-Investigators (US & Ethiopia)***

- Assume responsibility for the study in the absence of the Principal Investigator
- Supervise local workers, lab workers, nurses, local health agents, and other Proctor team members in the field to ensure conformity to study procedures
- Communicate with the program manager and principal investigator to ensure the execution of the study as per the protocol

*3.3.5 **WASH Expert (Emory University)***

- Serve as co-investigator and WASH technical advisor
- Provide technical assistance in fundamental behavioral interventions and qualitative methods
- Help design the formative research component of the study, including in-field training and analysis
- Help design the structured observations methodology, including in-field training and analysis

*3.3.6 **Statistical Analyst (Proctor Foundation)***

- Maintain database for all *collection and results-related data* for the study
- Monitor receipt of all census, treatment, and exam data after each collection activity
- Develop database for entry of costing data at the Carter Center, Addis Ababa
- Track and analyze process indicators including household WASH survey, spot check data, and structured observations
- Develop consistency checks in the data management—verify any inconsistencies or questions regarding data through communication with Study Coordinator
- Analyze and provide data regarding collection and results for study staff when needed, such as for publications or DSMC meeting
- Back-up all data appropriately
- Follow-up on any missing data or lab results

*3.3.7 **Microbiologist (Proctor Foundation)***

- Train laboratory technicians, especially the microbiologists at the Amhara Public Health Institute, for all lab procedures concerning the study: including Polymerase Chain Reaction (PCR) procedures, macrolide resistance testing, standard laboratory procedures for all tests, record-keeping, and quality control measures as per approved standards
- Verify equipment quality

3.3.8 Microbiologist/laboratory technician (Amhara Public Health Institute)

- Provide training review and supervise laboratory technicians for all lab procedures concerning the study, including PCR procedures
- Perform all macrolide resistance testing
- Follow standard laboratory procedures for all tests
- Keep records of all tests performed
- Maintain quality control measures as per approved standards
- Make sure all equipment is calibrated and maintained
- Maintain stock of laboratory reagents and supplies
- Pool and prepare all PCR samples, and process using Abbott RealTi*m*e assay for *Chlamydia trachomatis*, using the automated Abbott *m*2000 System
- Perform regular maintenance checks of the Abbott *m*2000 system, under direction of microbiologist
- Report PCR results to DCC
- Maintain cleanliness and maintenance of molecular biology laboratory and all equipment

3.3.9 Laboratory Assistants (Amhara Public Health Institute)

- Organize all study samples for PCR processing in at APHI
- Monitor correct receipt of study samples (using corresponding exam sheets) after each monitoring visit
- Prepare samples for PCR processing
- Organize work flow of PCR processing for laboratory technicians, in consultation with program manager and principal investigator
- Maintain all samples in storage freezers; create and maintain clear map of study samples for each freezer
- Process NP samples

3.3.10 Biostatistician (Proctor Foundation)

- With the Principal Investigator, create the Statistical Analysis Plan (SAP)
- Receive all study data and review for quality control purposes
- Ensure appropriate masking
- Prepare data analysis plan for annual DSMC meetings. Help analyze and prepare all presented data for DSMC meetings, DSMC reports, and all SWIFT study publications

3.3.11 Hygiene Cluster Coordinator: Local Staff, Amhara, Ethiopia

- Complete intensive on-site training from the Proctor Foundation and Carter Center
- Work closely with the Study Coordinator (TCCE) to ensure proper implementation of WASH components
- Responsible for overseeing 20 clusters
- Responsible for overseeing Hygiene Promotion Workers (HPWs)
- Conduct trainings for health extension workers, priests, and Health Development Army members
- Conduct monthly visits to all WASH communities

- ***Water:***
  - Help with identification of potential water point locations and management of the water point construction
  - Troubleshoot maintenance issues with Woreda Water Office or artisans
- ***Sanitation:***
  - Perform spot checks of latrine quality during monthly visits
  - Encourage households to build a latrine and keep it in good working order
  - Lead latrine promotion campaigns
- ***Hygiene:***
  - **Training of Teachers:** help conduct a training for teachers of primary schools in the study area; "train-the-trainers" model where teachers then implement activities for students
  - **Key messages:** Help conduct focus groups in clusters to identify the educational messages that will most motivate behavior change; work closely with Study Coordinator (TCCE) to help identify key hygiene promotion messages in hygiene promotion activities and appropriate forums for education
  - **Monthly workshops:** Supervise HPWs as they conduct monthly workshops in each cluster, with educational messages in appropriate local venues
  - **Spot checks:** Conduct spot checks on a random sample of 8 households with pre-school children at each monthly visit to monitor water, sanitation, and hygiene components

3.3.12 Health Promotion Workers (HPWs)

- Complete intensive on-site training workshop from the Proctor Foundation and Carter Center
- Work closely with the Hygiene Cluster Coordinator (TCCE) to ensure proper implementation of WASH components
- Responsible for WASH education in 1-3 clusters
- ***Water:***
  - Help troubleshoot maintenance issues
  - Report issues with water points
  - Liaise with Water Committee
  - Encourage increased water use for hygiene activities and use of safe water sources
- ***Sanitation:***
  - Perform spot checks of latrine quality during household visits
  - Encourage households to build a latrine and keep their latrine in good working order
- ***Hygiene:***
  - **House to house visits**: visits each household at least once per month to encourage positive WASH behavior change and improvements to hygiene infrastructure at the household level
  - **Monthly workshops:** conduct educational hygiene focused workshops in each cluster
  - **School curriculum**: work with school principal and WASH club leader to promote WASH in the schools and ensure that the schools are implementing the curriculum

3.3.13 ***Health Development Army***

- Local community members selected by woredas in Amhara, Ethiopia
- Assist with implementation of hygiene activities
- Complete trainings for WASH promotion
- Implement WASH promotion to 5 nearby households

3.3.14 ***Census Team***

- The Carter Center will organize and train local workers to perform the census.
- During the census, travel to enrolled clusters and obtain patient information as required for all census forms. The census will be performed with an electronic data capture system, with the following data inputs for each household/household member:
    - Name
    - Gender
    - Age
    - School(s) household utilizes
    - Water point(s) household utilizes
    - Health provider(s) household utilizes
    - GPS coordinates for each household
    - GPS coordinates for each safe water source in the study area
    - Presence or absence of usable household latrine and latrine-use
    - Presence or absence of washing station and soap
    - Information on hygiene behaviors (e.g., face-washing and latrine use)

3.3.15 ***Treatment Team***

- Complete training by TCCE Study Coordinator regarding proper treatment protocols and procedures
- Travel to enrolled clusters and administer study medication to the study subjects per protocol
    - Directly observe consumption of azithromycin
    - Record antibiotic coverage in census application
    - Return to households until they have distributed antibiotics to at least 80% of individuals
- Counsel and motivate participants for follow-up and monitoring visits
- Inform patients of available health care facilities and procedures in local health centers and hospitals
- Collect information on the nature of any Adverse Events experienced by study subjects, and report this back immediately to TCCE Investigators

3.3.16 ***Exam Team: Local workers, nurses, and lab technicians***

- At each exam phase, complete on-going training and certification in clinical examination and collection procedures
- Prepare all study-related materials before travel to study sites
- In each cluster, mobilize and identify all randomly selected participants

- Explain study purpose and procedure and obtain verbal consent for enrollment from each participant or participant guardian
- Under supervision of TCCE Study Coordinator and/or trained Proctor staff, perform clinical exam for each patient and collect all participant study samples according to protocol.
- Store and record all samples correctly for transport, organization, and processing
- Counsel and motivate patients for follow-up and monitoring visits
- Inform patients of available health care facilities and procedures in local health centers and hospitals
- Enter all data into the mobile application
- Collect information on the nature of any Adverse Events experienced by study subjects, and immediately report to TCCE Investigators

3.4 Policy Matters

3.4.1 ***Protocol Revisions during the Trial***

Any changes to the protocol made during the course of the study will be incorporated in the revised protocol and the Manual of Operations and Procedures (MOP), and recorded in a change log. Any new forms will be incorporated in addendum. The protocol changes should be submitted and approved by the IRB of both the collaborating centers and by the DSMC.

3.5 Presentations and Publications

All presentations and publications should include acknowledgement of the funding sources and give credit to the collaborating organizations and/or individuals involved.

3.5.1 ***Authorship Policy***

Acknowledgements will include grant source(s) and/or NIH grant(s), and the DSMC.

4. RESEARCH DESIGN

4.1 Study area

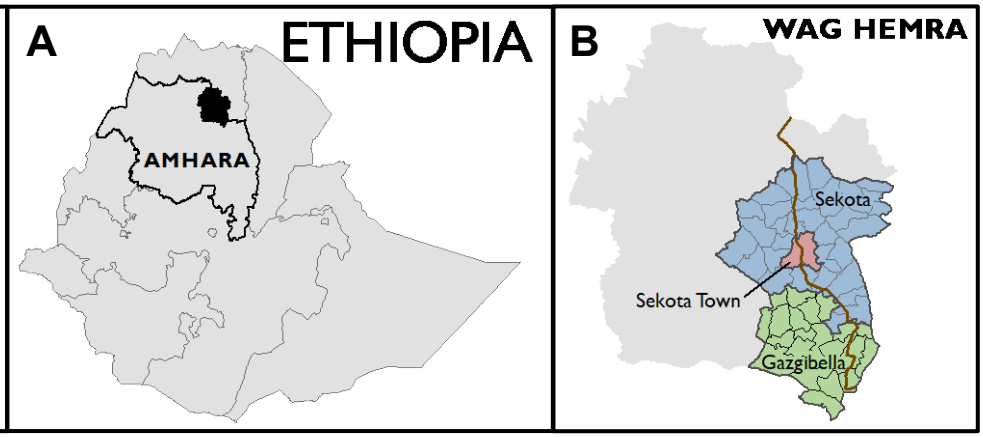


Figure 3. WUHA Study Site. Panel A shows the Amhara Region of Ethiopia and the WagHemra zone (shaded black). Panel B shows the 3 study *woredas* (Gazgibella, Sekota, and Sekota Town), the *kebele* borders, and the major road of the study area. We excluded the urban school districts in Sekota Town, as well as areas that are difficult to access.

SWIFT is being conducted in three woredas of the WagHemra zone of Amhara, Ethiopia: Gazgibella, Sekota, and Sekota Town (Figure 3). In Ethiopia, a *woreda* is a government-defined administrative subdivision that is in turn comprised of *kebeles*. WagHemra is arid and mountainous, and was chosen based on trachoma impact surveys performed by the Carter Center that determined the area had a high prevalence of trachoma and a low prevalence of several WASH indicators.

4.2 Randomization

4.2.1 ***Randomization Unit***

WUHA - School Districts: The randomization unit for this trial is the primary school district. We included 40 primary school districts from the 3 *woredas*. We excluded school districts in the 2 urban kebeles of Sekota, since urban areas have better access to water and sanitation and exhibit less trachoma than rural areas.[32,33] We chose the school district for two reasons. First, school children in Ethiopia have a considerable burden of trachoma, so units smaller than a school could be subject to contamination. Second, we wished to incorporate school-based hygiene promotion in the intervention, which requires a randomization unit of school district or larger. Within each school district, the best site for water point development was identified via geohydrologic survey, and the cluster of households within 1.5km of this potential water point was designated to receive the full package of WASH interventions as well as annual monitoring visits for trachoma. We call these households a study cluster.

TAITU - Villages/Gotts: The randomization unit for TAITU will be the village, which is called a gott in Amharic. There are 36 gotts enrolled in TAITU.

4.2.2 ***Treatment Arm Randomization***

After identification of the study clusters, we will conduct the baseline monitoring visit. Once the baseline visit has been concluded, the 40 WUHA study clusters will be randomized into the 2 treatment arms:

WASH (N=20) and delayed WASH (N=20). The 36 identified gotts for inclusion in TAITU will be randomized to targeted azithromycin (N=16), mass azithromycin (N=16), or delayed treatment (N=16).

By performing the randomization after the baseline monitoring, we reduce the possibility of differential outcome assessment at the baseline visit. The randomization sequence will be generated by the trial biostatistician in San Francisco using a random number generator, without stratification or blocking.

4.3 Eligibility requirements

4.3.1 ***Selection of study communities within clusters***

For WUHA, although we will randomize primary school districts to the intervention or control, we will actively perform the WASH interventions only in a limited group of households that are within 1.5km of a potential water site. In the spring of 2015, collaborators from Catholic Relief Services in Ethiopia performed a geohydrologic survey of the study area and identified sites that could potentially be developed as protected springs, hand dug wells, or shallow boreholes. We chose the best site from each of 40 school districts for inclusion in the trial, and censused all households within 1.5km of the potential water site.

4.3.2 ***Intervention eligibility:***

- **WUHA:** Within a study cluster, all households and all individuals living in these households are eligible for the WASH intervention.
- **TAITU**: All individuals are eligible for the intervention in the 16 MDA clusters. Within the 16 targeted study clusters, only those children aged 0-5 years who test positive for ocular chlamydia by PCR will be eligible for the antibiotic intervention.

4.3.3 ***Contamination***

Cluster-randomized trials are subject to contamination between clusters, which could weaken the effect of the study intervention. We reduce the chances of contamination by studying only a single cluster of households from a relatively large geographic area (i.e., school district), essentially creating a buffer zone around each study cluster. The chances of contamination are further reduced for household-delivered interventions since they are provided to households enumerated during the census. We have made no attempt to limit contamination from outside the study, which is theoretically possible since all 40 communities have continued to receive the usual health promotion from the government. The government program consists of two health extension workers per *kebele* who provide 16 different services, among them health education and promotion of hygiene and sanitation. We will contact all other non-governmental organizations known to be active in Amhara that provide WASH services(e.g., CARE, Save the Children, and WaterAid) and request that we coordinate activities to ensure that they either (1) postpone activities until the end of the study, or (2) build water points and conduct hygiene activities only in the intervention clusters.

*4.3.4 **Monitoring eligibility***

- **Population-based sample.** At each annual study visit, we will monitor the following groups:
  - For WUHA, we will monitor a random sample of children aged 0-5 years and a random sample of 30 children aged 6-9 years annually. We will also monitor a random sample of 30 individuals ≥ 10 years at baseline and month-36 in the WUHA clusters.
  - For TAITU, we will monitor all 0-5-year-old children and a random sample of 30 children aged 8-12.
  - Eligibility for each of these groups will be based on the previous census.
- **Longitudinal cohort.** The longitudinal cohort will consist of all children aged 0-5 years at baseline, followed annually until month 36 for the WASH trial, and followed until month 24 for the targeted azithromycin trial. 0-1-year-old children who are randomly selected for the population-based sample at months 12, 24, and 36 will be added to the cohort at subsequent visits.

4.4 Masking

Because of the nature of the interventions, it is not feasible to mask the study participants to treatment allocation. By not masking study participants, there is a chance that they will change their behaviors due to knowledge of their allocated treatment group. In the case of this trial, however, the entire purpose of the interventions is to change behaviors. Although there is a chance that individuals in the non-intervention communities will also improve their hygiene due to knowledge of their allocated treatment group, this is not likely—especially given the difficulty of causing behavior change even under optimal programmatic conditions. Because all interventions occur at the community level, study participants are surrounded by similarly treated individuals and therefore are not reminded of their treatment allocation very often, if at all. Moreover, we monitor WASH outcomes in both the intervention and non-intervention communities in order to assess the impact of the lack of masking on changes in hygiene behavior. We do not inform census workers or outcome assessors of the treatment allocation, although we acknowledge that this information would not be difficult to obtain.

The emphasis of masking efforts for the trial concerns the primary and secondary outcomes, which can easily be masked. All laboratory staff are masked to treatment allocation, as are the conjunctival photograph reviewers. All specimens collected in the field are labeled with a 5-digit random identification number. Photographic graders and laboratory staff have access only to this identification number, and not to any other identifying information for study participants.

We will also assess several key process indicators in a masked fashion from both intervention and control clusters (e.g., annual household survey, structured observations), but others in an unmasked way only in the intervention clusters (spot checks, focus groups).

5. STUDY INTERVENTIONS

5.1 WUHA Trial: WASH Uptake for Health in Amhara (Specific Aim 1)

The Carter Center Ethiopia is the principal implementing partner. The interventions have been informed by focus group discussions held in the study area as well as nearby areas. The Ethiopian study coordinator and the hygiene cluster coordinator are responsible for ensuring the fidelity of the intervention. The cluster coordinator had prior experience implementing WASH interventions for local nongovernmental organizations, and has close ties to local governmental health, water, and school officials. Each element of the package is described in detail below:

5.1.1 ***Water***

- **Community water point.** We worked with Catholic Relief Services (CRS) to install water points. CRS is a nongovernmental organization with extensive experience constructing water points in Ethiopia. CRS performed a geohydrologic survey and coordinated water point construction in each of the 20 WASH communities with the help of the local Ethiopian nongovernmental organization Water Action. Of the 20 water points, 13 were spring developments, 4 were hand dug wells, and 3 were shallow boreholes.
- **Water committee:** Interactive community participation is important for the successful implementation of a water point.[73] The Carter Center study coordinator and Catholic Relief Services worked with kebele leaders to identify a 5-person water committee for each water point, with at least 2 female members. This committee developed a plan for maintenance of the water point, including a payment system to subsidize repairs.

Figure 4. Household wash station in WUHA.

- **Household wash station.** During the initial focus group discussions conducted before the study, community members pointed out that one barrier to good face hygiene was the lack of a dedicated wash basin near the household. The ideal household wash station was identified as a jerry can with a faucet, similar to the types of facilities they saw in bigger cities. Based on this information, we provided all households in the intervention communities with such a jerry can (Figure 4), along with a mirror. Households were responsible for building a base for the jerry can out of locally available materials.

5.1.2 ***Sanitation***

- **Latrine promotion:** Current government policies do not allow the provision of building materials or direct assistance for building latrines, and so study activities are limited to latrine promotion at present. Study staff known as hygiene promoters (see below) are responsible for promoting latrine construction. Through door-to-door visits, hygiene promoters observe the presence and functionality of latrines and provide education about the value of a latrine as well as practical tips for construction.

*5.1.3 **Hygiene***

- **Targeted behaviors:** Hygiene education is most effective when confined to a few key messages, repeated in many different settings.[76] Although the WASH package could have a positive impact on many health outcomes (e.g., diarrhea, soil-transmitted helminths, respiratory infections), we focus on two behaviors likely to have the greatest impact on trachoma: (1) using soap and water to wash a child's face twice per day, and (2) consistently using latrines for defecation. Our messaging (e.g., times of day to wash the face, inclusion of soap, promotion of simple pit latrine, etc.) was based both on our initial focus group discussions and local government policies.

Figure 5. Example page of the household WASH book in WUHA.

5.1.3.1 Community-based hygiene promotion

- **Household WASH book:** During our initial focus group discussions in the study area, local government health extension workers showed us educational pamphlets for family planning that they found useful for education activities, and recommended that we create something similar for WUHA. What resulted was a 65-page full-color book that provided a roadmap for hygiene education for hygiene promotion workers and health development army members. The content of the book was based on many focus groups held with local educators, health officials, and water officials. Ethiopian artists provided the illustrations. The book was translated into the two languages spoken in the study area. A book was delivered to each household in the intervention clusters, to be kept for the duration of the study. Books that are lost or damaged are replaced annually.
- **Soap distribution:** The importance of soap was evident from the baseline focus group discussions. An American soap manufacturer (SoapBox Soaps, Washington, DC) donated funds to purchase 4 bars of locally produced bar soap to distribute to each household each month. The soap is purchased in Addis Ababa and transported by truck to the study site, where it is stored in one of two custom-built storage sheds in the study area.
- **Hygiene promotion workers (HPWs).** Twelve people were hired as HPWs to live in the study communities and perform door-to-door education activities. The HPWs use the WUHA household WASH book as a basis for interactive teaching, with the goal of covering all topics of the book with each household over the study period. The HPWs also use a standardized form to make hygiene observations at each household (e.g., the presence and use of latrines and wash stations), and use the results to focus their activities. HPWs report to the study's hygiene cluster coordinator. As part of SWIFT II, we will increase the number of HPWs to have one HPW assigned to each cluster, since this aspect of the intervention has proved to be quite popular among participants.
- **Non-study personnel**

- **Health Development Army (HDA).** This is a government-run program in which communities are divided into one-to-five networks, with one HDA household in charge of five other households. HDA members receive government training in various health topics, including hygiene. For WUHA, we perform annual supplementary training sessions on hygiene and encourage the HDA members to promote hygiene messages to the 5 other households in their network at least monthly using the WUHA household WASH book. HPWs check in with the local HDA members regularly.
- **Priests.** People in this part of Ethiopia are overwhelmingly Christian and devout. We perform an annual training session for all priests in the intervention communities and ask them to promote improved hygiene practices once per month at Sunday services.

5.1.3.2 School-based hygiene promotion

- Schools provide a convenient site for hygiene promotion and dissemination for several reasons. First, ocular chlamydia is primarily transmitted by children. Second, hygiene behaviors and habits are established in childhood. Third, parents identified school children as a potential vehicle for hygiene education during focus group discussions.
    - **Curriculum:** We developed a primary school hygiene curriculum in collaboration with the Carter Center and Ethiopian Department of Education that was based off a series of key informant interviews with teachers, principals, and health officials. The curriculum consists of 5 to 6 age-appropriate lesson plans per year for grades 1 through 4. Curriculum development was highly iterative, with many rounds of feedback from all stakeholders as well as thorough pilot-testing in our study area. The curriculum was designed to be interactive and student-centered, and to integrate well with the existing government curriculum.
    - **Teacher training:** a 3-day training is held before start of the school year each year. Germ theory and the general principles hygiene were reviewed. The lesson plans are also taught during this time.
    - **WASH clubs:** In this part of Ethiopia, children are required to participate in at least one extracurricular activity, and many schools have health or hygiene clubs. For WUHA, we provided training materials for WASH activities (songs, dances, dramas, community engagement activities) to existing WASH club leaders, and worked with principals of all intervention schools to ensure that WASH clubs were formed if they did not exist.

*5.1.4 **Mass albendazole distribution.***

The entire study area will be dewormed with a single mass albendazole distribution to all individuals aged 1 year and older (200mg for children aged 12-23 months and 400mg for individuals 24 months and older). The albendazole distribution occurred after the baseline study visit.

5.1.5 ***Hygiene Cluster Coordinator and HPW training***

After their selection, the hygiene cluster coordinator and HPWs will be trained at an intensive workshop led by the Proctor Foundation and Carter Center. We will review basic information about trachoma and its transmission, as well as the benefits of the WASH strategy for preventing transmission of trachoma. Although not the target of the current intervention, we will also include information about diarrhea prevention. We will make training as participatory as possible using various methodologies, including lectures, case studies, role play, and games. We will train the hygiene cluster coordinator and HPWs using a "train the trainer" paradigm, since this person will in turn be responsible for leading workshops in the study communities. We will use a "teach-back" model, where the hygiene cluster coordinator/HPW is asked to convey health promotion information back to the study coordinators, and the study coordinators provide feedback.[47] Before being certified, the hygiene cluster coordinator and HPWs will have to pass a written and oral examination in the local language, conducted by the TCCE study coordinator.

5.2 TAITU: Targeted Antibiotic Intervention for Trachoma in Under-5s (Specific Aim 2)

5.2.1 ***Conjunctival swabs (Targeted treatment clusters)***

We will collect conjunctival swabs on all 0-5-year-old children as described elsewhere in this document. Swabs from the targeted antibiotic trial will be expedited for processing at the Amhara Public Health Institute. Swabs will be processed for chlamydial PCR in pools of 5, with pools containing swabs from the same study cluster and age group (0-5 years and 8-12 years for TAITU). Any positive pools from the 0-5-year-old age group will be unpooled and processed individually. As described elsewhere in this document, swabs will be labeled with a random identification number in the field. This number will be linked with the study participant's census entry during swab collections in the field. The laboratory will provide to the Carter Center study coordinator a list of all random numbers from 0-5-year-old children and whether they are positive or negative for ocular chlamydia.

5.2.2 ***Identification of children to be treated (Targeted treatment clusters)***

The database's backend interface (Salesforce.com) application will automatically link the list of chlamydial PCR results provided by APHI to the census data. The study coordinator will generate a list of all children with positive chlamydial PCR results for each study cluster via Salesforce.com, and organize the treatment workers to administer directly observed azithromycin treatment to all children with a positive PCR result (20mg/kg, approximated with height-based dosing; children who are under 6 months or who have a known allergy will be offered a two-week course of topical tetracycline instead). The worker will document all children who receive antibiotic treatment. See subsequent sections in this document for details on azithromycin treatment. Note that all other individuals in the targeted treatment clusters (i.e., 0-5-year-olds without a positive chlamydial PCR test and all individuals ≥ 6 years) will not receive treatment for trachoma. We anticipate that the treatment will occur approximately 3 months after the baseline monitoring (i.e., 2 months to process conjunctival swabs, 1 month to organize treatment), although we will treat children as soon as the PCR results are available.

5.2.3 ***Additional rounds of targeted azithromycin treatment (Targeted treatment clusters)***

All children who have a positive PCR test at the baseline monitoring visit will receive three rounds of azithromycin treatment. We anticipate therefore that the treatments will occur at 3, 6, and 9 months after baseline, and then we would conduct a repeat monitoring visit at 12 months, after which the same treatment plan would be performed for year 2. These additional rounds of treatment will be performed by the treatment workers and coordinated by the Carter Center study coordinator and cluster coordinator.

5.2.4 ***Mass azithromycin treatment (MDA clusters)***

In the 16 study clusters randomized to mass azithromycin treatment, all individuals will receive a single mass azithromycin distribution 3 months after the baseline and 12-month monitoring visits. The MDA will occur at 3 months to align with the first targeted treatment. Oral azithromycin, 20mg/kg for children and 1g for adults, will be offered to all households identified on the preceding census. Antibiotics will be distributed by treatment workers and organized by the Carter Center. Individuals with a known macrolide allergy, babies under 6 months, and pregnant women will be offered a 2-week course of twice daily ophthalmic tetracycline ointment (2 tubes) in lieu of the azithromycin treatment.

6. INTERVENTION MONITORING AND EVALUATION

6.1 Spot checks

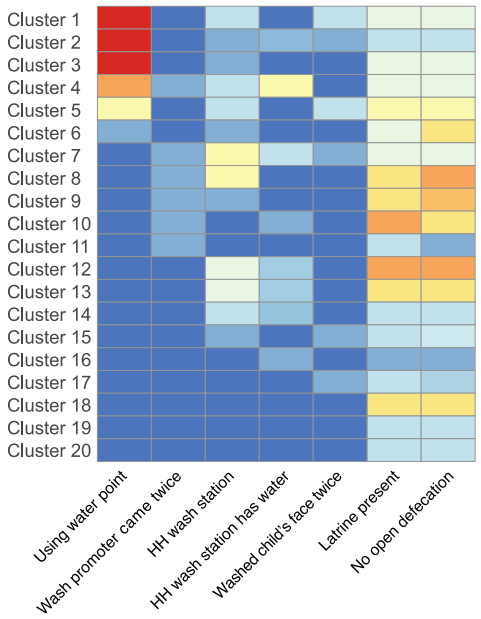


Figure 6. Spot check example. March 2017 spot check of 20 WASH communities, visualized as a heat map (blue=100% of households; red=0%).

The study cluster coordinator conducts several annual rounds of spot checks annually in the WUHA intervention clusters. At each spot check, the hygiene cluster coordinator assesses the usability of the water point and checks in with the school teachers in the cluster to review their implementation of the hygiene curriculum. The hygiene cluster coordinator also visits a random sample of 8 households with pre-school children and documents the presence of a wash station and its functionality (e.g., presence of water in the container and soap), the presence of a latrine and its functionality (e.g., whether walls and a roof are present), and evidence for latrine use (e.g., trodden latrine path, fresh feces in the pit). The content of these structured spot checks is based on our experience in Ethiopia and the experience of others who have conducted similar surveys.[34,52] The cluster coordinator enters all data into a smartphone using an offline REDCap mobile application, and then syncs the data later that evening. Electronic data capture is important, since it allows us to analyze and share the data more rapidly. As shown in Figure 6, the spot check data are visualized as a heat map in order to easily identify the hygiene gaps in each community. The hygiene cluster coordinator then conveys this information to the hygiene promotion workers during their monthly phone call and they brainstorm ways to improve the deficiencies.

6.2 Household survey at annual census

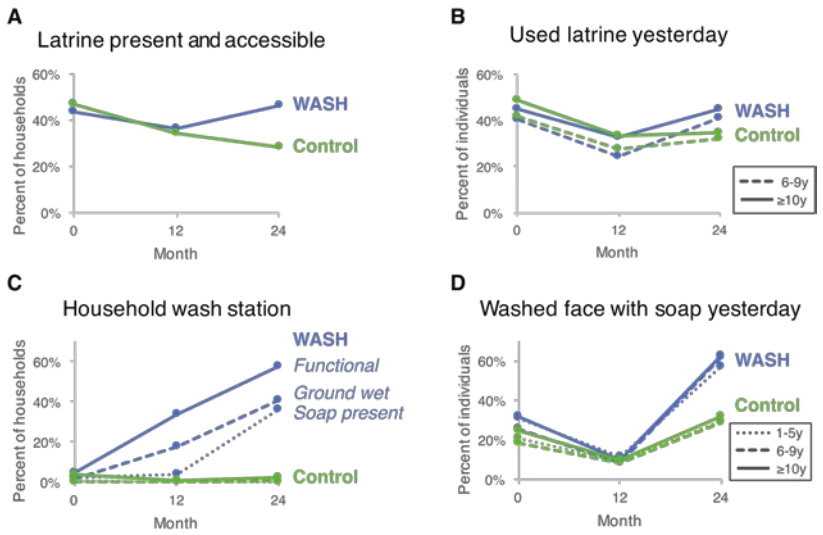
We perform a household survey in a random sample of 33% of households during the census. Census workers are not informed of the study purpose or the randomization allocation. The survey questions capture both self-reported hygiene behaviors as well as objective observations and has been crucial for understanding the uptake of the intervention and for guiding remediation efforts. Figure 7 shows the results from key survey items from the first three censuses (December 2015, 2016, and 2017). The month 24 results show that communities randomized to the WASH intervention are more likely to have a household WASH station and latrine, and household members from WASH communities are more likely to report having washed their face and used the latrine in the past day. Behavior changes in the WASH arm were most evident between the month 12 and 24 visits, which is likely due to two main reasons. First, hygiene behaviors are notoriously difficult to change, and hygiene interventions are thought to require long periods of time before they can influence behavior. Second, our WASH intervention has many components, and was not fully implemented until the month 12 visit, meaning that the period of

time between month 12 and 24 is likely a more accurate reflection of its impact. Given how difficult it is to change hygiene behaviors, these results suggest a real and marked difference between the two arms, and provide strong evidence that our intervention is being implemented as intended.

6.3 Focus Group Discussions

Hygiene promotion must take into account the local context. Barriers to changing hygiene practices and factors that motivate changing behavior are site-specific. We will build off previous successful hygiene promotion efforts that have relied on formative research and community participation to guide health messaging priorities.[35,41-43]

Figure 7. Household survey at month 0, 12, and 24. The survey was performed on a random 33% of households from 20 WASH clusters and 20 control clusters during the annual census.



The study team will hold focus groups periodically throughout the trial. The team will choose a representative sample of communities and they will use a discussion guide to lead the focus groups in a discussion about barriers and motivations for hygiene practices, especially as they relate to face washing and latrine use. Sessions will last no more than 2 hours. We will target all appropriate stakeholders. These will include at least the following groups:

(1) Health Development Army (men and women)
(2) Community Leaders
(3) Strong hygiene performers
(4) Poor WASH performers
- Poor hygiene performers are a group of people that will be identified by HPWs as those individuals who have poor hygiene behaviors. This may mean that they do not own or use a latrine, that they do not have a wash station at the household, that their compound is not kept clean, etc.

The focus groups will be analyzed during the study visit during which they are conducted in order to inform the WASH intervention.

We performed a round of focus group discussions in 5 intervention communities in June 2017 and in October 2018 to assess the impact of the intervention and also to help guide our intervention moving forward. We learned that community members highly valued the hygiene promotion workers and soap distribution. We probed for reasons that latrine construction was low; participants reported that besides people's habits being difficult to change, the costs of latrine construction were a major barrier.

We also learned that households appreciated the WASH book, that it was regularly used during home visits, and that the illustrations were easily understandable.

6.4 Structured observation

Although we will capture the presence of hardware (e.g., improved water points, latrines, washing stations) during the annual census, this trial also requires detailed assessment of hygiene behaviors (e.g., face-washing and latrine use). Moreover, these assessments must occur in both treatment groups in order to fully assess the impact of the WASH package. Various methodologies exist to measure hygiene behaviors, including qualitative techniques, surveys, and structured observation.[34-36] Each has limitations. For example, structured observations are vulnerable to reactivity, wherein those being observed change their behaviors simply from the process of being observed.[37] Nonetheless, structured observation has been used routinely and is well accepted by the WASH sector. We will hire and train a team of local individuals to perform full-day structured observations of face-washing and latrine behaviors in both the WASH and control communities when the intervention is fully rolled out. Study staff will obtain consent from the household and then sit in an area of the household where the wash station, other water sources, and latrine can be observed. The observer will document use of the latrine as well as face- and hand-washing behavior for each household member, and will also note facial hygiene in the children of the household (i.e., nasal/ocular discharge, presence of flies). The observer will document all water use, including water used for cooking, cleaning, and bathing, and perform a spot check of latrine and cleanliness indicators, including the presence of human and animal feces in the compound. Each household will be observed for about 22 hours, from mid-afternoon on one day to mid-afternoon the following day, in order to be present at the household in the morning and evening, since this is when the majority of hygiene behaviors take place. Because structured observation is time-consuming and labor intensive, we will select a random sample of 5 households per cluster for observations, chosen from the preceding census. Only households with a child aged 0-9 years will be eligible for inclusion. We anticipate hiring 10 staff members, and completing data collection over a 6-week period, immediately following the census. Dr. Freeman, who is the WASH expert for this trial, has experience with the design and analysis of structured observations, and will lead the implementation of this part of the study.

7. CENSUS

We will perform a house-to-house census of the selected study clusters at baseline, prior to randomization. We will perform census updates at months 12, 24, and 36. The Carter Center will organize and train local workers to perform the census. The Carter Center has experience in conducting large censuses in Ethiopia, as they conduct censuses each year before mass azithromycin treatments. We will use an electronic data capture system for the census activities, similar to a pilot study we conducted in Ethiopia in 2011 and similar to the electronic system we are currently using for a trial in Niger funded by the Bill & Melinda Gates Foundation (OPP 1032340, Tom Lietman, PI). This system utilizes tablet computers to record all census data. Census workers record the name, sex, and age of each household member, the primary school(s) that any children attend, and the GPS coordinates of the house. In a random one-third of households, we perform a household survey to gauge self-reported socioeconomic status, access to water, and hygiene behaviors, and also perform observations of household latrines and wash stations. Census updates are performed annually thereafter, approximately one month prior to the exams.

7.1 Equipment

Each census worker is given the following equipment:

1. Tablet computer: Huawei Ascend mate 2 or Mi A2 (one)
2. Battery Pack: Anker dual port charger (one)
3. Charging cord (from charger to electrical socket); (one)
4. Connecting cord (from computer to charger); (one)
5. Trident protective case (one)
6. Sealable canvas bag to hold all equipment (one)

7.2 Mobile Application

A custom-made software application, which runs on the Android platform, will be used for data collection in the study. Census workers will enter all data directly into the application. The application is capable of using Amharic letters, so census workers will type the names of all study participants in Amharic.

Census workers will be trained how to upload and download data. At the initial census, there was no pre-existing data so downloading is unnecessary. At a follow-up censuses, the pre-existing data from the most recent census will be downloaded in the morning before going to the field. The data will be updated in the field, and then uploaded when the teams return back to town at night.

The data will be uploaded to a secure online server on a daily basis so that the study team can monitor census progress in real time.

7.3 Training and Supervision

The study coordinators from the Proctor Foundation and Carter Center will run a 3-day training workshop at the woreda health office for all census takers to train them in the following topics:

- General use of the tablet computers
- Entering data into the mobile application
- Acquiring GPS coordinates with the device
- Re-charging the device

After the in-class training, census workers are accompanied in the field for training in a practice village and for the first few days of actual data collection to ensure that quality data is consistently collected.

One team leader is selected from each 3-person census team, and that person is responsible for team supervision, ensuring good coverage of the cluster, uploading, and daily charging.

7.4 Charging Devices

The team leader will be responsible for charging all devices from their team each night. This will be done at the supervisor's home. As a backup, the study coordinator or cluster coordinator will be available to charge the devices at a hotel.

8. ANNUAL MONITORING (EXAMINATION) PROCEDURES

We will perform annual monitoring visits in all study clusters. At each visit, we will collect samples on a subset of the population in the study cluster, as described below.

8.1 Sampling strategies

We will employ two different sampling strategies for the trials.

8.1.1 ***Population-based sample***

The population-based sample will consist of an age-stratified cross-sectional sample at each monitoring visit. Eligibility will be based on the most recent census, which will be completed annually several weeks before monitoring visits. We will sample a separate cross-sectional sample of individuals at each monitoring visit, so that individuals may or may not be sampled at successive visits. We employ this strategy in order to be able to obtain an unbiased population-based estimate of infection and other outcomes at each visit of the trial.

Table 1: The number of individuals in each age stratum will depend on the particular outcome:

	WUHA			TAITU	
Test	**0-5 years⁺**	**6-9 years⁺**	**≥ 10 years⁺¹**	**0-5 years**	**8-12 years⁺**
Conjunctival swabs	30	30	30[1]	All	30
Eye exam/photo	30*	30	30[1]	All*	30
Face photo	30*	30		All*	30
Stool sample	30[2]	30[2]		--	--
Nasopharyngeal swab	15⁺¹			15⁺¹	
Dried blood spot	30*	30*[3]	30*[4]	All*	
Anthropometry	30*			All*	

*Longitudinal sample (month 12: all 0-6, month 24: all 0-7, month 36: all 0-8-year-olds)
⁺ Random sample
[1] NP swabs only collected at baseline and endline visits
[2] Stool sample data only collected at 0 and 12 months
[3] Blood spot data for 6-9-year-olds captured at month 0 and 36
[4] Blood spot data for ≥ 10-year-olds captured at month 36

8.1.2 ***Longitudinal sample***

For outcomes such as anthropometry, clinically active trachoma, and facial cleanliness, we are interested in assessing changes within an individual. Therefore, we will also employ a longitudinal sampling strategy for these outcomes. Specifically, we will longitudinally assess the entire cohort of 0-5-year-old children included in the population-based sample at baseline (30 children for WUHA and all children for TAITU), with 0-12-month-olds who are randomly sampled in the population-based sample added to the cohort annually. The cohort will grow each year, as new babies are added in and as the older members age. At

month 12, the cohort will consist of 0-6-year-olds. At month 24, the cohort will consist of 0-7-year-olds. At month 36, the cohort will consist of 0-8-year-olds. For the outcomes of face photographs, conjunctival photographs, dried blood spots, and anthropometry, we will monitor the cohort over the course of the study visits.

8.2 Preparation

8.2.1.1 Registration lists

Before examinations can begin, registration lists with the sampled populations must be made. This is done in Salesforce.com, keeping the following points in mind:

- Make sure the census is complete. Check in the app to make sure all households have been completed.
- Make sure the subphase is set to *Examination* on the *Geographic Work Unit* object.
- Navigate to the *Geographic Work Unit* and click on *Create Morbidity Study*.
- Generate a pdf to give to the mobilizer(s) for participant recruitment
- Before going to the field, download the registration lists on each tablet/smartphone. This requires internet connectivity.

8.3 Training

*8.3.1 **In-Country Training***

Before the start of the study, local health workers will be selected to complete study fieldwork and will undergo a series of rigorous trainings and certification in clinical grading and swab collection. At the start of each successive collection phase, examination team members (nurses and lab workers from the Ministry of Health and general workers from WagHimra) will participate in three-day training in WagHimra. Two days will take place in the classroom and will cover use of the application, examination procedures, and sample collection procedures. The final day of training will take place in a practice cluster. All exam workers must pass a test to participate in the exam data collection. The swabbers must pass a grading exam to participate in the roll of swabber/grader. The Principal Investigator and Study Coordinators will offer ongoing training and supervision to all exam workers in the field throughout the SWIFT study monitoring visits.

8.4 Exam Team

*8.4.1 **Mobilizers***

The Carter Center study coordinator will hire a community member from each monitored community to mobilize the selected participants and ensure a high turnout for the monitoring visit. This person will also select the location for the monitoring visit and remove any hygiene posters or other materials that could unmask the outcome assessment.

8.4.2 ***Outcome Assessors***

In collaboration with woreda health officials, the Carter Center study coordinator will hire lab workers, nurses, and general workers from WagHimra to perform all outcome assessments. These workers will receive a 3-day training session at the beginning of the monitoring visit to review all study procedures. They will be masked to treatment allocation.

8.5 Registration Station

8.5.1 ***Personnel***

One registration worker is needed. This person must be familiar with the mobile data collection application and comfortable using a tablet/smartphone, and speak the local language.

8.5.2 ***Procedures***

The Registration Station is always the first station that study participants should visit. It should be placed in a visible area. The registration worker performs the following:

- Download the registration list in the mobile application; normally this will be done before the team arrives in the field, in a location with good internet connectivity.
- Identify the participant on the list of potential participants from the mobile application, and click the name to enter the participant's registration page.
- Verify demographic information (name, age, sex, mother, father).
- Note the background color of the participant's name in the mobile application. This color corresponds to the exam station that they must visit.
- Place the next available sticker from the roll of random number stickers onto a bracelet with the color the matches the participant's background color in the application. This number is the participant's registration number.
- Write the participant's full name, age, and sex, onto the bracelet.
- Have the participant hold the bracelet below their chin, and take a photo on the participant's registration data page such that a photograph is taken of the face that also includes the person's name and registration number.
- Fasten the bracelet around the participant's wrist. This should be done fairly tightly, so that the bracelet cannot be easily removed.

8.6 Swab station

8.6.1 ***Personnel***

In collaboration with district health officials, the local study coordinator will hire staff from the local health offices. Field staff will receive a 3-day training session at the beginning of the monitoring visit to review all study procedures, and will be masked to treatment allocation. Each swab team will be comprised of 3 people:

- Tuber: must be meticulous and detail-oriented
- Swabber/Grader: must have good clinical skills

- Photographer: must be familiar with the mobile data collection application, photography, and be a fast learner

*8.6.2 **Scanning registration code***

The photographer will use the bar code scanner function on the mobile application to scan the registration code on the participant's bracelet.

8.7 Clinical Photography

*8.7.1 **Clinical Photography of the Conjunctiva***

Four specific team members will be designated as photographers for the entire trial in order to ensure high quality photographs by an experienced photographer. The photographer will take photographs of the conjunctiva with a handheld Samsung NX digital SLR camera with a macro lens (1:1). Conjunctival photography causes no damage to the eye, is well tolerated by children, and is a standard clinical procedure at UCSF. Clinical photography will be performed before conjunctival swabbing. Photography is incorporated in the mobile application; photographs will be taken under a specific child's record and therefore automatically associated with the child's unique identifier. Photographs are stored on the memory card in the camera. A compressed version of the photograph is synchronized to the study database along with the other data. The full-size version of the photograph will be transferred to an external hard drive each week. Batteries are charged each night; each camera bag has at least 1 spare external charger.

*8.7.2 **Photography Protocol***

- Equipment needed: camera
- Miscellaneous equipment: Extra battery, media card reader, lens filter, extra media card

8.7.2.1 Camera Setup

- Model
  - Samsung NX, which has the advantage of running on Android, and therefore has seamless integration with the mobile application
- Lens
  - Samsung 60mm ƒ/2.8 macro lens
- Settings
  - The photographer does not need to adjust the camera settings because the settings are automatically set by the mobile application according to the following parameters:
- White balance- automatic
- Aperture Priority
- F-stop: ƒ/32
- ISO 400
- Manual focus

- Flash: on (fill-in)
- EV (brightness): 0

8.7.2.2 Photograph Procedure

- Place participant into position that will allow maximum stability; standing, sitting or "head-clamp" position. Employing a village volunteer to help is very useful.
- Extend lens fully, to the 1:1 position
- Image is brought into focus by changing the working distance, not by turning the lens. This is because the lens is fixed in its manual setting. The working distance is approximately 20 cm from the eye with our current settings
- Take minimum of 2 photos. If there is any doubt of the quality of the photo while the patient is in position it is better to continue to take more photographs before the patient is allowed to leave.
- Check photos before allowing child to leave. If they are not acceptable, repeat procedure. Only stop if the patient or guardian requests that we stop, or if is deemed impossible, even with further attempts.
- If the child cannot be photographed for some reason, it does not affect the eligibility of the child. A notation is made on the child's record of why the photograph cannot be taken, but no replacement is sought. This is expected to be a rare event.

8.7.2.3 Photo Troubleshooting Guide

Note all camera settings are permanently embedded in every photo that is taken and can be viewed with the camera or with any standard commercial photo-viewing software (e.g., Adobe Photoshop; Photomechanic, Nikon View, Canon, etc.)

Photos too DARK:

Make sure flash is functioning. If first photos in series are acceptable and then they gradually become less exposed (darker) it might be because the battery is gradually losing power during the session. Note that the flash reaction time increases as the battery power decreases.

- Image not centered
  - Movement of child or camera (hold child's head between knees of "helper"; stabilize camera with second hand)
- Reflection artifact
  - Move camera slightly between first and second photos to achieve different angle; gently dab conjunctiva with swab- must be done at periphery to avoid creating inflammation

8.7.2.4 Face Photograph Procedure

Face photographs are taken with the same camera and lens, using settings defined by the mobile application (same as above, except an F-stop of ƒ/11). The photographer stands approximately 1-2

meters from the participant and moves the lens until the face fills the frame of the photograph, with only a small amount of space between the chin/ears/cranium and the edge of the photograph.

8.7.3 ***Conjunctival Swab Collection Procedures***

8.7.3.1 *Gloving*

Any hand that will touch a participant's skin must be gloved for the examination. The examiner will put latex gloves on both of their hands prior to touching a participant's skin, and a new pair of gloves will be used for each participant. Purell® Instant Hand Sanitizer will be available for hand sanitization.

8.7.3.2 *Equipment*

- Swabbing/tubing: tubes, swabs, labels, tube box, cooler, ice, metal container for swabs, aprons, garbage bags, chucks, hand sanitizer
- Grading: 2.5X loupes, penlight
- Photography: camera, extra batteries, lens wipes

8.7.3.3 *Examination Position*

Young children: The examining position to be used in the field for young children will be the classic pediatric ophthalmic examination technique. With the aid of a helper seated directly opposite to the examiner, the child will be positioned with his/her head between the examiner's knees, with the child's face looking upwards toward the examiner. The legs of the child will be straddled across the helper and the arms held gently across the child's chest. Care should be taken to keep the child's eyes above the level of the examiner's knees, in order to properly take the conjunctival swab.

Older children/adults: For examining adults and older children, the participant should stand or sit facing the seated examiner, such that the participant's eyes are at the examiner's eye-level.

8.7.3.4 *Everting the Upper Eyelid*

For all participants, only the right upper eyelid will be examined; the only exception to this is if the right eye is difficult to examine due to eye disease or injury, in which case the left eye will be examined. In order to avoid passing contamination from the child into the eye, once the examiner dons a new pair of gloves, their gloved hands should not be used to position the child. The examiner uses their fingertips to grasp the central portion of the participant's upper lid eyelashes. The upper lid is then everted, using a finger of the examiner's other hand (or the end of a sterile swab) as a fulcrum, positioned superior to the tarsal plate. The everted lid is held in place by the examiner's non-dominant hand holding the eyelashes against the orbital rim, thus keeping the examiner's dominant hand free for swabbing the participant's tarsal conjunctiva later.

8.7.3.5 Trachoma Grading

The examiner uses the 2.5X magnifying ocular loupes to assess the tarsal conjunctiva of the everted upper right eyelid. The examiner will grade the conjunctiva according to the World Health Organization Simplified Trachoma Grading Scale, as shown in Table 2. A hand-held penlight will be used by the examiner for illumination of the conjunctiva at all times; the only substitute for this is direct sunlight. The examiner tells the trachoma grade to the photographer, who records the grade into the mobile application on the Samsung camera. Before being allowed to grade in the field, each grader must complete a training workshop and pass a photographic test with a kappa of 0.6 or greater relative to a gold standard grade, where the gold standard is the consensus grade from a panel of 3 expert graders,

Table 2: WHO Simplified Trachoma Grading Scale

Definitions of the WHO Simplified Trachoma Grading Scale
TF (Trachomatous Inflammation – Follicular): the presence of five or more follicles in the upper tarsal conjunctiva.
TI (Trachomatous Inflammation – Intense): pronounced inflammatory thickening of the upper tarsal conjunctiva that obscures more than half of the normal deep tarsal vessels.
TS (Trachomatous Scarring): the presence of scarring in the tarsal conjunctiva.
TT (Trachomatous Trichiasis): at least one eyelash rubs on the eyeball.
CO (Corneal Opacity): easily visible corneal opacity over the pupil.
Clinically active trachoma: *defined as either TF or TI. If WHO guidelines recommend that TF alone is the most appropriate sign to follow, then we will easily be able to report this.*

8.7.3.6 WHO Simplified Trachoma Grading Card

The WHO simplified trachoma grading card will be used as the reference standard for grading trachoma in the field. The card shows color photograph standards of each trachoma grade, clear descriptions of each grading category, and the standard abbreviations for each trachoma grade.

8.7.3.7 Procedures

In this trial, we will collect 1 swab. The examiner uses a separate pair of gloves for each study participant. We will store the swabs in microcentrifuge tubes without transport media.

- The tuber opens a Dacron swab by the bottom end and makes it available to the swabber.
- The swabber passes the Dacron swab firmly over the right everted upper tarsal conjunctiva three times, rotating 120ºC between each pass.
- The tuber holds a tube with the cap open
- The swabber deposits the swab in the tube, and snaps off the shaft of the swab. This normally does not require counterpressure with the tube cap.
- The tuber firmly screws closed the cap. The tuber checks to make sure that the swab is at the bottom of the tube and taps the tube until the swab falls.

- The tuber places the tube in the tube box within the cooler. Tubes are put in chronological order of swabbing, filling a horizontal row, starting with the top left-hand corner of the box.
- The tuber closes the cooler in between swabs.

8.7.4 ***Two sets of control swabs will be taken in the course of examinations:***

Negative "air" swabs: a swab will be waved in the air, taking care not to contaminate the swab. This will be done at the beginning and end of the work day. The tube codes are scanned into the mobile application in the designated field.

Duplicate swabs: The mobile application will randomly select 2 participants per community for a duplicate swab.

8.7.5 ***Transportation and Storage of Conjuntival Samples***

In accordance with the Abbott RealTi*m*e protocol, swab samples taken in the field will be transported on ice in a closed, insulated container, and then transfer them within 8 hours to a -20°C freezer at a local health center. Swabs will be shipped within 4 weeks to the APHI, where they will be stored at -20°C before being processed.

8.7.6 ***Nasopharyngeal Specimen Collection***

At months 0 and 36, we will collect nasopharyngeal swabs in 15 randomly selected children aged 0-5 years per community. We selected this age group because pre-school children in sub-Saharan Africa have been shown to have high rates of pneumococcal carriage.[107-109]

- The tuber will select an NP swab and open the swab sachet in a sterile manner (revealing only the tip of the swab shaft, with the swab head itself remaining sterile deep within the sachet).
- Immediately after completion of the conjunctival eye swab collection (and if necessary, control swab collection), the examiner will remove the NP swab from the sachet and place the tip down the participant's nasopharynx.
- The examiner will quickly rotate the swab 120° three times back and forth, and then remove the swab from the nose.
- The examiner will place the swab in a tube containing 1.0 mL of STGG (skim milk, tryptone, glucose, and glycerin) media. The tuber will cut the handle off using sterile scissors (cleaned with alcohol pads between participants). The tuber will close the cap of the STGG tube with the swab immersed.
- We will keep the swabs on ice in the field and then in a -20°C freezer as described above for the conjunctival swabs. The Amhara Public Health Institute will process the swabs using media selective for *Streptococcus pneumoniae*, and then test for antibiotic resistance to erythromycin, penicillin, tetracycline, and clindamycin using the Kirby Bauer disc diffusion assay.

8.7.7 ***Materials for NP Collection***

- Nasopharyngeal Swabs: Specimens will be collected using sterile, individually-wrapped pediatric calcium alginate swabs with a malleable plastic swab shaft for patient comfort and safety

- Nasopharyngeal Sample Tubes: All field samples for DNA testing will be collected into sterile 2.0ml microcentrifuge tubes, manufactured by RPI®.
- Cooler Bags with Frozen Ice Packs: Insulated cooler bags will be used to carry samples to and from the field. In addition, frozen gel ice packs designed to thaw slowly will be used to maintain the temperature in the cooler bags during transport.
- -20°C Freezer: A standard -20°C freezer will be used strictly for the storage and freezing of ice packs and samples. This freezer is kept in a locked room on the grounds of the Health Center, which is under 24-hour security guard supervision.
- Latex Gloves: latex examination gloves will be used to perform nasopharyngeal examinations. Each glove will be used once and never shared between participants. Used gloves will be collected in a trash bag and incinerated at the local Health Center incineration facility.

*8.7.8 **Transportation and Storage of Nasopharyngeal Samples***

The nasopharyngeal swab samples in skim milk-tryptone-glucose-glycerin (STGG) will be initially stored in the field using an insulated storage bag filled with Fisher brand ice gel packs and then transferred along with the conjunctival swabs first to a -20 freezer in WagHimra and then to Bahir Dar.

8.8 Anthropometry

Using methods we have already used in several studies in Ethiopia and Niger, we will measure the height, weight, and middle upper arm circumference (MUAC) of the longitudinal cohort of 0-5 year-old children.[96,106] Each index will be measured in triplicate and the median measurement used for analysis. We will use a Shorr Board to measure height, Seca 874 floor scale to measure weight, and MUAC tapes to measure MUAC. Anthropometric measurements in previous studies have had excellent repeatability, and we have found such assessments to be feasible and inexpensive. We will urgently refer any children with severe acute malnutrition, defined as MUAC <11cm, to a local health clinic for treatment.

*8.8.1 **Observe***

When the participant presents for examination, first examine him/her for signs of malnourishment:

1. **Kwashiorkor:** look for edema (or swelling); thin, sparse, or discolored hair; and skin with discolored patches that may crack and peel.
2. **Marasmus:** look for severe wasting; the appearance of 'skin and bones;' and a face that looks like an old man's.
3. **Pedal edema:** look for swelling due to excess fluid in the foot. Press the child's foot with your thumb. If the foot is swollen and the indentation remains after you press it, the child has edema.

Each anthropometry team should have a laminated sheet with photographs demonstrating typical examples of these signs.

*8.8.2 **Measuring and Recording Guidelines***

Measurements are taken to the nearest 0.1 cm (1.0 mm) for height/length and MUAC, and 0.01 kg for weights.

*8.8.3 **Measuring Length and Height***

A lightweight measuring board will be used to measure the participant's height to the nearest 0.1 cm. Height will be assessed with a ShorrBoard (ShorrBoard®, Shorr Productions, LLC, Olney, MD, USA). Depending on a child's age and ability to stand, measure the child's length or height.

Length: If a child is less than 2 years old, measure recumbent length. Select **L** to indicate that length was measured. A child's length is measured lying down (recumbent).

Height: If a child is aged 2 years or older and able to stand, measure standing height. Select H to indicate that height was measured. Height is measured standing upright.

If the child has braids or hair ornaments that will interfere with length/height measurements, remove them if possible. Check that any shoes or socks have also been removed.

Whether measuring length or height, the mother/guardian is needed to help with measurements and to soothe and comfort the child. Explain to the mother the reasons for the measurements, and the steps in the procedure. Answer any questions she might have. Show her and tell her how she can help you. Explain that it is important to keep the child still and calm to obtain the best measurement.

Table 3: Shorr Board Set-Up

1) Remove from bag.
2) Stand the Shorr Board upright. You can step on the base of the board to keep it stable.
3) As you face the board, turn the non-removable bolt counterclockwise to release the extension piece. Note: the bolt remains attached to the back of the extension piece – do NOT remove it.
4) Slide the extension piece into the top end of the main board and fasten the clasp on the back of the board. Make sure the clasp is fastened properly.
5) The auto-lock sliding head/footpiece is stored in the base of the main board and can be moved up and down the length of the measuring board. It should stay in place on its own wherever you position it.
6) The measuring board must be placed against a firm surface for standing height (e.g. wall, table, tree, etc.). Make sure the board is stable. If necessary, place items such as small rocks underneath the height board to stabilize it during the measurement.
7) Clean the equipment with alcohol swabs at the beginning of each day.

8.8.3.1 Measure Length

I. Cover the length board with a chuck (or another absorbent, disposable material) for hygiene and for the baby's comfort.

II. Explain to the mother that she will need to place the baby on the length board and then help to hold the baby's head in place while the measurement is taken. Show her where to stand when placing the baby down (i.e. opposite you, on the side of the length board away from the tape). Also show her where to place the baby's head (against the fixed headboard) so that she can move quickly and surely without distressing the baby.

III. When the mother understands your instructions and is ready to assist: Ask her to lay the child on his back with his head against the fixed headboard, compressing the hair.

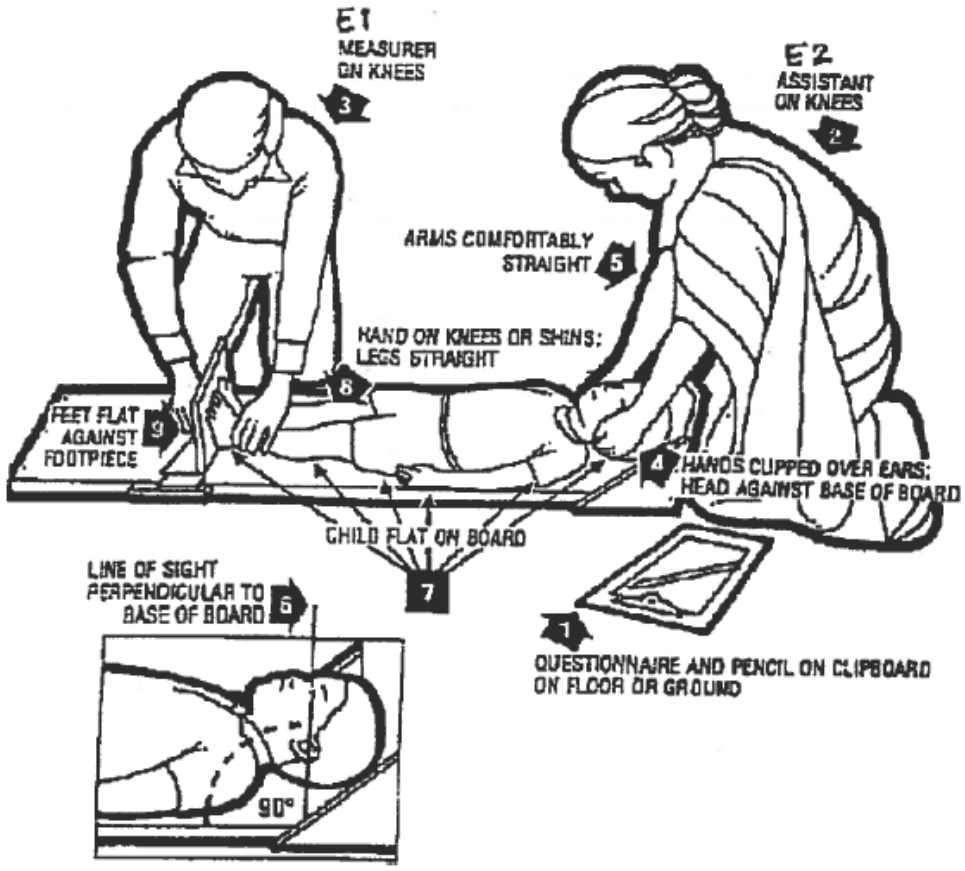
IV. Quickly position the head so that an imaginary vertical line from the ear canal to the lower border of the eye socket is perpendicular to the board. (The child's eyes should be looking straight up.) Ask the mother to move behind the headboard and hold the head in this position.

V. **Speed is important.** Standing on the side of the length board where you can see the measuring tape and move the footboard:
   a. Check that the child lies straight along the board and does not change position.
   b. Shoulders should touch the board, and the spine should not be arched. Ask the mother to inform you if the child arches the back or moves out of position.
   c. Hold down the child's legs with the one hand and move the footboard with the other. Apply gentle pressure to the knees to straighten the legs as far as they can go without causing injury or distress. **Note: it is not possible to straighten the knees of newborns to the same degree as older children. Their knees are fragile and could be easily injured, so apply only minimum pressure.**
   d. If a child is extremely agitated and both legs cannot be held in position, measure with one leg in position.
   e. While holding the knees, pull the footboard against the child's feet.

VI. **Upon reading the measurement, the examiner will clearly call out the number to the recorder.** Record the child's length in centimeters to the last completed 0.1 cm. (1.0 mm).

VII. Keeping the child in place, release the sliding footboard, and prepare to repeat the measurement. Re-position the child for a second and third measurement.

Figure 8: Measuring length



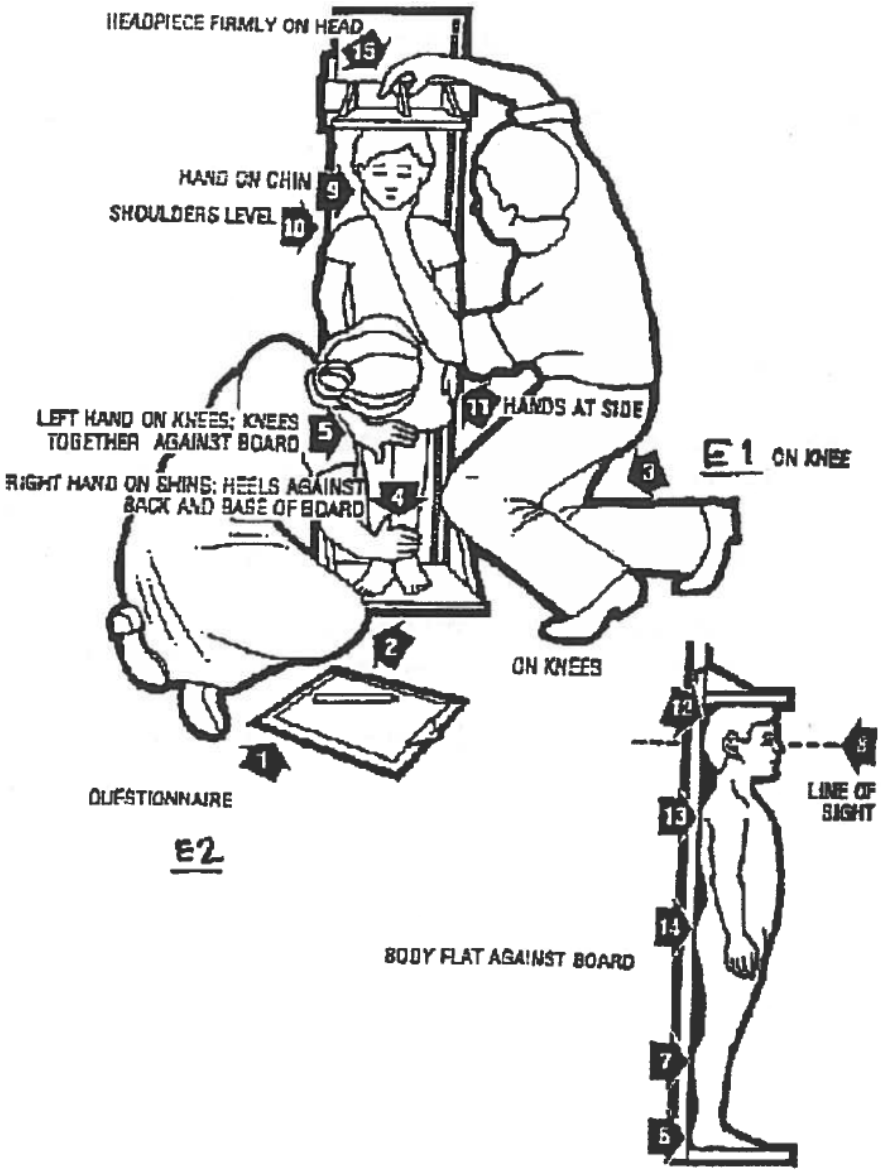
8.8.3.2 Measure Standing Height

1. Ensure that the height board is on level ground.
2. Working with the mother or another helper, and kneeling in order to be at the level of the child:
    a. Help the child stand on the baseboard with the weight of the child evenly distributed on both feet. The heels of the feet are placed together with both heels touching the base of the vertical board. Place the feet pointed slightly outward at a 60-degree angle.
    b. The back of the head, shoulder blades, buttocks, calves, and heels should all touch the vertical board. Arms should hang freely by the sides of the body with the palms facing the thighs. **Note: Standing with all body parts touching the board may be difficult for some children, in which case, help the child to stand on the board with one or more contact points touching the board.**
    c. Ask the mother to hold the child's knees and ankles to help keep the legs straight and feet flat, with heels and calves touching the vertical board. Ask her to focus the child's attention, soothe the child as needed, and inform you if the child moves out of position.
    d. Position the child's head so that a horizontal line from the ear canal to the lower border of the eye socket runs parallel to the baseboard.

e. Ask the child to inhale deeply and to stand fully erect without altering the position of the heels. If necessary, push gently on the belly to help the child stand to full height.
f. Still keeping the head in position, use your other hand to pull down the headboard to rest firmly on top of the head and compress the hair.

3. **Upon reading the measurement, the examiner will clearly call out the number to the recorder.** Record the child's height in centimeters to the last completed 0.1 cm (1.0 mm).
4. Keeping the child in place, release the sliding headboard, and prepare to repeat the measurement. Re-position the child for a second and third measurement.

Figure 9: Measuring standing height



8.8.4 **Dismantling the Shorr Board**

- Stand the board upright: face the board and step on the base with one foot to keep it stable.
- Slide the head/foot piece into the base of the main board.
- Release the clasp on the back of the extension piece and remove it. Push the clasp FLAT against the extension piece.
- To attach the extension piece to the main board, turn the front of the extension piece inward and place it against the front of the main board. Make sure that all sides of the extension piece are straight and in line with the main board.
- Push on the bolt that is on the back of the extension piece and screw it into the main board.
- Put the board back inside of the carrying case for storage until your next use.

8.8.5 ***Measuring Weight***

The SECA 874 scale or suitable alternative will be used to weigh infants and children to the nearest 0.01 kg (Seca 874 flat floor scale, seca GMBH & Co. Kg, Hamburg, Germany). Infants and young children can also be weighed simultaneously with their parent or guardian by the unique "mother-baby" function (parent or guardian is weighed and then the infant or child is weighed while held by the parent).
Explain to the mother that we want to weigh her child to see how he or she is growing. If she has a baby or a child who is unable to stand, she will hold the child on the scale. If the child is 2 years or older, the child will be weighed alone. Children should be wearing only light clothing, no shoes, no hair ornaments, and no jewelry. Explain that the child needs to remove outer clothing and shoes in order to obtain an accurate weight. If the baby is wearing a diaper, the diaper should be removed. If any heavy clothes remain on the child, make a note in the *Comments* section.

8.8.6 ***Procedures***

- Remove scale from bag.
- Be sure that the scale is placed on a flat, hard, even surface. All 4 legs of the scale should make contact with the ground surface, without wobbling.
- Turn the power on the scale when you are ready to begin weighing.
    - Note: When **batt** appears in the display, you should change the batteries. Remove the old batteries and insert 6 new batteries.
- Press the start key with no load on the scale. The scale is ready for use when it sets to 0.00.
    - If necessary, switch the weight display to KG: hold down the 2 in 1 key for about 3 seconds.
    - Press the start key with no load on the scale. The scale is ready for use when it sets to 0.00.
- Weighing the child alone
    - The child removes their shoes and any heavy clothing
    - Ask the child to stand in the middle of the scale. Once on the scale, the child must stand still. The HOLD function is automatically activated for weights over 1.5 kg/3.3

lbs. The display flashes until a stable weight has been measured. The display is then frozen until the next weighing operation.
    - Note: If the child jumps on the scale or won't stand still, you will need to use the tared weighing procedure instead.
- **The positioner will clearly call out the child's weight to the recorder.** Record the child's weight to the nearest 0.01 kg.

Have the child step off and back onto the scale
- **The positioner will clearly call out the child's weight to the recorder.**
- Record the child's weight to the nearest 0.01 kg.
- Repeat one more time

2-1 feature: **If the child is unable to stand on the scale**, you will use the 2 in 1 weighing function (called *tared* weighing). The **2 in 1** function enables the weight of babies and small children to be determined while an adult holds them.
- Ask the adult to stand in the middle of the scale without the child. She should remove any long garments, as these can cover the display and also lead to variable measurements.
- After the mother's weight appears on the display, tell her to remain standing on the scale. Press the **2 in 1** key to activate the function.
- The scale stores the weight of the adult and the display returns to zero. When 0.00 and NET appear in the display, hand the child to the adult. The scale will determine the weight of the child. Once the value is stable for about 3 seconds, the weight is measured. Note: If a mother is very heavy (e.g. more than 100 kg) and the baby's weight is relatively low (e.g. less than 2.5 kg), the baby's weight may not register on the scale. In such cases, have a lighter person hold the baby on the scale.
- Repeat the measurement 2 more times. Note that only the baby needs to be removed from the scale; the mother may remain on the scale the entire time.

- To turn off the **2 in 1** function, press the **2 in 1** key. The **2 in 1** function remains on until you press the **2 in 1** key again, or until the scale switches off automatically.
- If several children are to be weighed consecutively with the same adult holding the babies, it is important that this person's weight does not change (e.g. due to a piece of clothing being removed/added).
- The scale automatically turns off after 2-3 minutes

8.8.6.1 Scale Calibration

We have shown that the weight measurements captured from the Seca 874 do not change over time, even in field conditions.[8] In order to monitor the calibration of the scales over time, each team will weigh a 5 kg test weight at the beginning and end of the day. This measurement will take place at the site of the scale storage so that the weights do not need to be carried to the field.

8.8.7 ***Measuring MUAC***

The child's MUAC will be measured at the midpoint of the right arm between the tip of the shoulder and the tip of the elbow, to the nearest millimeter using non-stretch MUAC tapes produced for clinical studies by Johns Hopkins University[53] and interpreted according to WHO guidelines.

I. First, **find the midpoint of the upper arm**. Have the child stand up straight with feet together, and the right arm bent 90 degrees at the elbow, palm facing up. The examiner is positioned behind the child.
   a. The most upper edge of the posterior border of the acromion process of the scapula is located and marked.
   b. Hold the zero end of the measuring tape at this mark and extend the tape down the back of the child's arm to the tip of the olecranon process (the bony part of the mid-elbow).
   c. The examiner reads the measurement aloud to the recorder.
   d. Keeping the tape in position, locate the spot which is half the distance from the acromion to the olecranon processes (i.e. the midpoint of the upper arm).
   e. The recorder will mark the midpoint on the back of the child's arm.

II. To **measure mid-upper arm circumference**, have the child stand up straight with the arms relaxed at the sides. The examiner will stand facing the child's right side. The measuring tape is placed around the upper arm at the marked point.

III. Wrap the tape around the arm, pulling it to lie flat against the surface of the skin. Be careful not to pull the tape too tightly (to compress the skin).

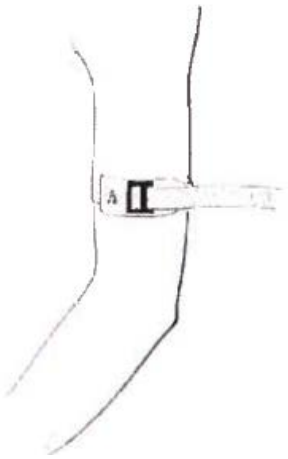
IV. Read the number which is between the two arrows on the window on the MUAC tape. Make sure that the lines inside and outside the window are completely aligned (that the line inside the window is not tilted). **Upon measuring, the examiner will clearly call out the number to the recorder.** Record to the nearest 0.1 cm (0.1 cm = 1 mm).

V. Keeping the child in place, release the MUAC strip, reposition, and measure for a total of 3 measurements.

Figure 10: Measuring MUAC

8.8.8 ***Materials for Anthropometry***

I. ShorrBoard
II. Seca scale
III. MUAC strips (2)
IV. Chucks and alcohol swabs
V. Pens and sharpie markers
VI. Trash bags
VII. Extra set of AA batteries (6)
VIII. Laminated sheet showing examples of malnutrition



8.9 *Stool Samples*

There are two separate collections for the STH study – 1) preserving stool in **SAF** to count eggs and parasites; and 2) storing stool in 5% potassium dichromate solution for **PCR** processing. Children will still be asked for just one sample, and study personnel will prepare two separate tubes. One tube will be transported to the Amhara Public Health Institute for processing and examined for the presence of the major soil transmitted helminths (*Ascaris lumbricoides*, *Trichuris trichiura*, and *Ascaris lumbricoides*). The other tube will be sent to Smith College for PCR analysis for *Necator americanus*, *Ancylostoma duodenale*, *Trichuris trichiura*, *Ascaris lumbricoides*, and *Strongyloides stercoralis*.

There are 4 steps to follow at the stool collection station:

I. Stool Sample Collection
II. Sample Preparation (SAF)
III. Sample Preparation (PCR)
IV. Waste Disposal

8.9.1 ***Stool Specimen Collection***

1. Place a plastic bag of child's s potty and give to the study participant.
2. Instruct the child to defecate in the bag on the potty, remove and twist the top of the plastic bag and return with the potty and bagged stool sample.
3. Remind the caregiver/child to bring the specimen immediately to the sample collection station.
4. Advise participant to urinate before collecting the stool so that they do not get any urine in the stool sample.
5. Give the child and/or caregiver hand sanitizing wipes when they return with the sample.
6. Check that the sample is sufficient amount. If there is not a sufficient amount ask the caregiver or child if they can produce more. If not, follow the instructions for next-day sample collection.
7. *Next-day sample collection*
    1. If a child cannot produce a sample by the end of the study visit day write the child's age, gender, and random number on the paper bag and plastic bag.
    2. Instruct the caregiver to have the child defecate in the bag, tie the bag up and place in the paper bag for pick-up the next day.

8.9.2 ***Sample Preparation (SAF)***

1. Wearing fresh gloves, label the SAF tube (10 ml) first with the random number and weigh the content of the tube to balance the weight prior to mixing with stool sample.
2. Using applicator stick, pick stool sample equal to size of 1 gram and mix it thoroughly with the SAF solution. Register the consistency and the weight of stool that you added.
3. Close the tube tightly
4. Seal tube with parafilm and shake tube vigorously
5. Place tube in SAF tube box (keep in shade)

8.9.3 ***Sample Preparation (PCR)***

1. Wearing fresh gloves, label a 2ml tube with the random number
2. Add .5 ml of fresh stool to the tube
3. Using a pipette, fill the other half of the tube (about .5ml) with the 5% potassium dichromate solution leaving room in the tube to mix the mixture
4. Close the tube lid tightly
5. Seal tube with parafilm and shake tube vigorously
6. Place the tube in the PCR tube box from left to right. The tube box should be located in cooler, and the cooler should be closed between each addition. The cooler should be in the shade.

8.9.4 ***Waste Disposal***

1. Discard the remaining stool sample, pipette, used applicator sticks in the trash bag.
2. At the end of the day/field work all waste has to be disposed appropriately (incinerated at a health post).

8.9.5 ***Materials for Stool Specimen Collection (pending feedback from Steve Williams)***

I. Child potty
II. Wooden Tongue depressor (dowels)
III. Digital specimen scale
IV. Stool specimen tube containing 10ml SAF preservative
V. 2ml tubes for PCR preservation
VI. Applicator stick
VII. Alcohol wipe
VIII. Fine tip permanent marker
IX. Trash bag
X. Gloves
XI. Batteries
XII. Random number sticker roll
XIII. Sanitizing hand wipes

8.10 ***Dried Blood Spots***

The gold standard microbiological test for detecting ocular chlamydia infection is a nucleic acid amplification test of a conjunctival swab. However, new serological tests being developed by the United States Centers for Disease Control and Prevention (CDC) are promising diagnostic modalities for monitoring of trachoma elimination. A positive test indicates a previous exposure to chlamydia as opposed to current infection; antibodies remain detectable even after a mass azithromycin treatment.[95] Preliminary testing in a hypoendemic setting in Tanzania has shown that serologic results in 1-3 year-old children is correlated with the prevalence of ocular chlamydia infection in a community,

suggesting that the serology results in young children may provide information about the level of transmission.[96] Current serologic methods are expensive, but the CDC is currently developing a much simpler and less expensive lateral flow assay (LFA) for use with eluted dried blood spots.[97,98]

In a setting where ocular chlamydia transmission has been eliminated, the youngest children will not be exposed to chlamydia and therefore should not have a positive serological test. The serological test in young children may therefore be a sensitive measure of chlamydial transmission within a community. Furthermore, we will use the blood spots to test antibody responses to other diseases of interest including helminths, intestinal bacterial pathogens, and intestinal protozoal pathogens. These tests are still being developed, but we anticipate that they will have been optimized by the time of the conclusion of this trial. Therefore, we will also collect dried blood spots from all children aged 0-5 years in the population-based sample each year. We will lance the index finger of each child and express 4-5 dried blood spots onto a TropBio filter paper. Filter papers will be labeled with a random number identification sticker and stored in the -20 freezer for later processing in Atlanta.

*8.10.1 **Steps for blood spot collection***

- Inform the mother that her child's finger will be pricked to obtain blood to test for trachoma, malaria. anemia, and other diseases. Describe the finger prick procedure, reassure her, and answer all questions. The blood specimen should be collected as described below to minimize the discomfort of the child and to ensure sufficient blood volume collection.
- Label the filter paper with the corresponding random number sticker.
- Prepare the disposable lancet. Make sure to use a new disposable lancet for each child. **Do not** re-use lancets.
- Position the child for the finger stick. Make sure that the child's hand is warm and relaxed. Hold the child's thumb, middle, or ring finger between your left thumb and finger
- Gently stimulate blood flow towards the puncture site by lightly squeezing the child's thumb or finger from the top of the knuckle towards the fingertip
- Disinfect the finger with alcohol wipe.
- Prick the side of the thumb/fingertip
    - For the best blood flow and least pain, prick the side of the thumb/fingertip, not the center.
- Wipe away the first 2 drops of blood with a new sterile cotton ball
- Place a droplet of blood from the finger onto **four-five** of the six circles
    - Leave one circle blank.
    - Touch the side of the circle
    - Fill circle completely.
    - **Note:** Allow blood to ooze out on its own**. Do not** squeeze forcefully avoid "milking" as it may dilute the blood with tissue plasma.
- Grip the filter paper on the side without small circles. Carefully slide the filter paper onto a pencil to air dry for at least an hour. There should be about 1 cm in between each sample. Secure the pencil into a Styrofoam surface in a cardboard box.
- When dry, place each blood spot paper into an individual small plastic bag while wearing gloves

- Combine all bagged samples in a larger community bag. Add desiccant packets
  - Label large bag with:
    - Label large bags:
    - SWIFT "visit number"
    - Cluster name
    - Number of samples collected
    - When were samples collected: (e.g. 19JAN2019)

*8.10.2 **Risk minimization***

- It is important to handle all specimens with care to minimize risk of infection
  - Wear gloves: New gloves must be worn for each child.
  - Clean spills: In the event of a blood spill or splash, clean immediately with disinfectant and wipe with absorbent material.
  - Disposal of sharps: All lancets must be disposed of properly in sharps containers.

*8.10.3 **Materials for Blood Collection***

- Gloves
- Disposable lancets
- Alcohol wipes
- Cotton balls
- 10% household bleach to clean spills
- Absorbent material for spills (cotton)
- Sharps container
- TropBio circular cards
- Small zip plastic bags
- Desiccant packs
- Large Ziploc bags (handful)
- Materials for drying apparatus: 12 sharpened pencils, Styrofoam, empty cardboard box

9. OUTCOME ASSESSMENT AND LABORATORY PROCEDURES

9.1.1 Photographic Reading Center

The Reading Center for grading of conjunctival and face photographs will be based at Gondar University. Three graders will independently grade each photograph, masked to treatment allocation. We will choose the consensus or median grade for all outcomes. We will assess inter- and intra-observer agreement with kappa statistics.

- **Facility/Equipment.** A dedicated room with a two computer monitors will be used to grade photographs. Full-screen photographs will be read at 26 inches with the shades drawn and lights off.
- **Naming Conventions.** The Proctor data analyst and program manager will be responsible for organizing the photographs taken during the study. The photos are stored on Salesforce, and they are labeled with an "attachementID" code. The program manager will download the photos and their metadata including the photo code, the phase they were taken during, and the participant's master record that they belong to. The photos will be uploaded to the offline program and linked to their metadata for grading.

9.1.2 Grading of clinical conjunctival photographs

Six ophthalmology residents will grade the photographs at the conclusion of the study, masked to treatment allocation. We will present graders all photographs taken from a phase for one individual. Graders will grade the set of photographs together for photo quality, follicles, inflammation, scarring according to the grading scale described in table 4. We chose this grading system because it provides enhanced granularity of grades compared to other grading scales, allowing for detection of more subtle differences in clinical trachoma. Graders will also compare the initial and final photographs of the set and document whether the final photograph has less, more, or equivalent follicles and inflammation compared with the initial photograph. Note that each set of photographs will have between 1 and 4 photographs, depending on how many monitoring visits the individual attended. We use photographic grades as the co-primary outcome of the trial since high quality photographs have good agreement with in-field grades, and offer the benefit of a masked outcome.[99,100]

Table 4: Questions for conjunctiva grading

Questions for Conj-grading-swift-036:

<table>
<tr><th>Question</th><th>Responses</th></tr>
<tr><td>1. Photo Quality</td><td>• 1 – Good (Default)
• 2 – Poor but acceptable
• 99 – Ungradable</td></tr>
<tr><td>2. Photos all of one eye</td><td>• 1 – All of one eye (Default)
• 2 – Includes other eyes
• 3 – Includes faces</td></tr>
<tr><td>3. Number of follicles on central tarsal conjunctiva</td><td>• 0
• 1
• …
• 14
• 15 or more
• 99 – Ungradable</td></tr>
<tr><td>4. Trachoma Inflammation</td><td>
<table>
<tr><th>Score</th><th>Structure not visible for >50%</th><th>Papillae</th><th>Description</th></tr>
<tr><td>5</td><td>Trunks</td><td></td><td>Trunks visible ≤ 50%</td></tr>
<tr><td>4</td><td>1st branches</td><td></td><td>Trunks visible > 50%, but not 1st branches</td></tr>
<tr><td>3</td><td>2nd branches</td><td></td><td>1st branch visible > 50%, but not the 2nd branch</td></tr>
<tr><td>2</td><td></td><td>> 50%</td><td>2nd branch visible > 50%, and papillae on > 50%</td></tr>
<tr><td>1</td><td></td><td>≤ 50%</td><td>2nd branch visible > 50%, and papillae on ≤ 50%</td></tr>
<tr><td>0</td><td></td><td>None</td><td>2nd branch visible > 50%, and no papillae</td></tr>
<tr><td>99</td><td></td><td></td><td>Ungradable</td></tr>
</table>
</td></tr>
<tr><td>5. Trachoma Scarring</td><td>
<table>
<tr><th>Score</th><th>Scarring</th><th>Shortening/ Distortion</th><th>Involving</th><th>Description</th></tr>
<tr><td>0</td><td>None</td><td>None</td><td></td><td>None</td></tr>
<tr><td>1</td><td>Fine, scattered</td><td>None</td><td></td><td>Fine, scattered scars</td></tr>
<tr><td>2</td><td>More severe</td><td>None</td><td></td><td>More severe scarring but NO shortening or distortion</td></tr>
<tr><td>3</td><td>Severe</td><td>Yes</td><td>≤ 50%</td><td>Severe scarring involving ≤ 50% WITH shortening or distortion</td></tr>
<tr><td>4</td><td>Severe</td><td>Yes</td><td>> 50%</td><td>Severe scarring involving > 50% WITH shortening or distortion</td></tr>
<tr><td>99</td><td></td><td></td><td></td><td>Ungradable</td></tr>
</table>
</td></tr>
<tr><td>6. Notes</td><td>Include your notes here. (Optional)</td></tr>
</table>

9.1.3 ***Assessment of Facial Cleanliness***

The ophthalmology residents will grade the face photographs for each child. The photos will be organized and uploaded in the same way as the conjunctiva photos. The photo set (1 child's face photos at 1 phase) will be graded on photo quality and the presence or absence of the following items: dust or dirt on the face, food on the face, dry secretions around the eyes, wet secretions around the eyes, dry secretions around the nose, and wet secretions around the nose. The number of flies on the face will also be counted. The grader will be masked to treatment allocation. Note that this is a novel way to determine facial cleanliness, and should result in complete masking of observers.

9.2 Specimen Collection for Microbiological Tests

Samples will be collected with reference to age, gender, household, and study cluster, but participant names will not be included in laboratory records. Samples will thus not be associated with the individual's name, but with a 5-digit random identification number, masking laboratory personnel and preventing identification of the individuals infected. Lab results will not be available for weeks if not months, typically after the average duration of an ocular chlamydial infection.

9.2.1 ***APHI Lab: Abbott RealTime Assay***

Laboratory testing is the current standard of care for the identification of *C.trachomatis* infections in the U.S. After collection, all samples will be processed the APHI and will be filled with 1 ml of M4RT media, and tested for *C. trachomatis*.

Swabs will be processed with the Abbott RealTi*m*e assay for *Chlamydia trachomatis*, using the automated Abbott *m*2000 System, which is already in use at APHI. The RealTi*m*e assay targets the cryptic plasmid of *C. trachomatis*. The assay has been shown to be highly sensitive and specific for the diagnosis of sexually transmitted *C. trachomatis*, with sensitivities exceeding that of the nucleic acid amplification test used in most recent trachoma studies (Roche AMPLICOR).[59,60]

9.2.2 ***Processing of Samples for PCR***

Samples are handled as per Abbott RealTi*m*e sample processing protocol, with the following modifications:

- Samples are boiled for 10 minutes at 100°C. Boiling of samples is an accepted treatment method to remove substances that may be inhibitory to the PCR amplification process.
- Samples are pooled

9.2.3 ***Procedure for Masking Samples***

The PCR results will be recorded according to the random number assigned to the sample, thus masking the lab since the lab workers do not have access to Salesforce in order to link this number with the child who provided the sample. Once the sample is processed, the analyst will then link the sample random number to the child's record on Salesforce to reveal the test results by cluster.

9.2.4 ***Procedure for Pooling Samples***

We will increase the efficiency of chlamydial testing by combining swabs from the same age stratum and same community into pools of 5 random swabs for processing. An internal control will be run with each pool to rule out the possibility of PCR inhibitors. Any inhibitory pools will be re-tested, and if still inhibitory, the swabs will be individually re-tested. If PCR from any pool is equivocal, then all swabs from the pool will be tested individually. While samples will necessarily be diluted in this process, this is not thought to affect the sensitivity of the test.[61] We will test individually all swabs from positive pools in the 0-5 year age stratum and estimate the community prevalence of chlamydial infection as the proportion of positive swabs. We will estimate the community prevalence of infection in the 6-9 and ≥10 (WUHA) year age strata using maximum likelihood estimation, similar to our previous trials: the number of individual swabs with the maximum likelihood of having resulted in the observed pooled results will be chosen as the estimate for that village.[16,62]

In order to pool the conjunctival samples in the lab, the microbiology lab staff at APHI will assign a new pool ID number for every sample, and samples will be stored at -20°C freezer until PCR testing (if not processed that day).

9.2.5 ***Quality Control***

1) A *C. trachomatis* (+) control and a *C. trachomatis* (-) control (targeting 136 base pairs of a pumpkin gene) is included in each test run of the Abbott RealTi*m*e assay.
2) To test the effect of sample processing, a known positive sample is processed and tested in each test run. (This control is helpful when testing large numbers of negative samples.)
3) An internal control intended to identify specimens that contain polymerase inhibitor is run routinely on each sample. The internal control helps identify false negative results.

9.2.6 ***Quantification***

For every 0-5-year-old who tests positive for chlamydia, we will also run PCR for the beta actin gene on the same sample, in order to normalize the quantity of chlamydial DNA to the amount of the specimen. Quantitative results from the Abbott system are given in terms of a decision cycle (DC) number. We will generate a ratio of the DC number of the chlamydial DNA to the DC number of the beta actin gene and use the resulting ratio as the chlamydial load.

9.2.7 ***Laboratory Results Reporting***

All lab results will be kept in computer files as well as uploaded to Salesforce by the analyst. The principal investigator and the DSMC will be updated regularly on the progress of the lab work throughout the course of the study.

9.2.8 ***Assessment of ocular chlamydia***

Qualitative PCR for chlamydial DNA. Swabs will be processed with the Abbott RealTi*m*e assay for *Chlamydia trachomatis*, using the automated Abbott *m*2000 System, which is already in use at the APHI. The RealTi*m*e assay targets the cryptic plasmid of *C. trachomatis*. The assay has been shown to be highly sensitive and specific for the diagnosis of sexually transmitted *C. trachomatis*, with

sensitivities exceeding that of the nucleic acid amplification test used in most recent trachoma studies (Roche AMPLICOR).[101,102] We will increase the efficiency of chlamydial testing by combining swabs from the same age stratum and same community into pools of 5 random swabs for processing. An internal control will be run with each pool to rule out the possibility of PCR inhibitors. Any inhibitory pools will be re-tested, and if still inhibitory, the swabs will be individually re-tested. If PCR from any pool is equivocal, then all swabs from the pool will be tested individually. While samples will necessarily be diluted in this process, 5-pooling has been shown to not significantly affect the sensitivity of the test.[103] We will unpool all positive pools in the 0-5 year age stratum and estimate the community prevalence of chlamydial infection as the proportion of positive swabs. We will estimate the community prevalence of infection in the 6-9 (WUHA), ≥10 (WUHA), and 8-12 (TAITU) year age strata directly from the pooled swabs using maximum likelihood estimation, similar to our previous trials: the number of individual swabs with the maximum likelihood of having resulted in the observed pooled results will be chosen as the estimate for that village.[19,104] Note that we are currently working with APHI to perform chlamydial testing, so the swab storage, transport, and processing protocols are already in place.

Quantitation of chlamydial load. We will assess the infectious load for all individual specimens from 0-5-year-old children who test positive for chlamydia. Load will be determined relative to a reference housekeeping gene (beta actin) using the Abbott RealTi*m*e assay, and expressed as the ratio of chlamydia to this housekeeping gene. We chose beta actin because this is a cytoskeletal structural protein that is ubiquitous and not thought to vary its expression.[105] Note that we are currently gaining experience performing quantitative PCR with the Abbott system at APHI.

9.3 Macrolide resistance testing

9.3.1 ***Specimen Collection for Macrolide Resistance Testing***

Nasopharyngeal samples will be collected with reference to age, gender, household, and cluster, but participant names will not be included in laboratory records. Samples will thus not be associated with the individual's name, but with a 5-digit random identification number, masking laboratory personnel and preventing identification of individuals.

9.3.2 ***Methods***

The nasopharyngeal swab for pneumococcal resistance testing will be transported and processed using standard microbiological techniques at APHI. We will store swabs in STGG medium, keeping swabs on ice in the field and then in the -20°C freezer as described above for the conjunctival swabs. APHI will process the swabs using media selective for *Streptococcus pneumoniae*, and then test for antibiotic resistance to erythromycin, penicillin, tetracycline, and clindamycin using the Kirby Bauer disc diffusion assay.

9.3.3 ***Procedure for Masking Samples***

The microbiologist and all lab staff will be masked to the development-team of origin.

9.3.4 ***Quality Control***

We will follow the standard quality control methods already in place at the laboratory. For example, lab staff will perform positive and negative growth controls for all media, and positive controls for all stains at a pre-determined schedule.

9.3.5 ***Laboratory Results Reporting***

All lab results will be kept in computer files as well as in hard-copy form by the Microbiologist.

9.4 Dried blood spots

We use Trop-Bio filter paper for dried blood spots in WUHA. Each paper has 6 ears, and we fill 5 ears with a drop of blood.

We ship the dried blood spots to Dr. Diana Martin at the CDC, who processes them for pgp3 and CT694 using the Luminex platform. The Luminex assay results are reported as a quantitative value (median fluorescence intensity minus background; MFI-BG); these results can be dichotomized based on the results of a receiver operating characteristic (ROC) curve from a set of known positives and negatives run on the same bead coupling. Serologic results provide evidence for exposure to chlamydial infection, and thus may be an especially sensitive metric of transmission.

10.CLINIC-BASED CASE FINDING

We will perform clinic-based case finding at all health posts serving the study clusters. Eligible health clinics will be identified by interviewing local community leaders during the census and woreda health officials. The cluster coordinator will take photographs of the health post log books. A data entry worker will use the phtographs to enter the data into a REDCap form. Data will include age, gender, community, diagnosis, and treatment. Diagnosis of infection or treatment with an antimicrobial will be considered an infectious disease. We will collect data on all individuals who live in a community containing a study cluster. We will retrospectively collect data for a full calendar year before the start of the study in order to provide baseline values.

11.COSTING

We will perform both a short-term and long-term cost effectiveness analysis (CEA) from the societal perspective. For the short-term analysis, we will perform a trial-based cost effectiveness analysis using community-level data from the clinical trial. For the long-term analysis, we will create a decision model to estimate the costs and effectiveness after the conclusion of the study, and determine the cost effectiveness at varying time horizons. For each analysis, we will report the incremental cost effectiveness at a given time point, with a range of uncertainty. The research plan adheres to guidelines for the design and analysis of trial-based and decision analytic cost-effectiveness analyses.[57,58]

11.1 Assessment of costs

11.1.1 ***Trial-based CEA***

We will prospectively collect cost data for all interventions in the WASH clusters during the study period, using the WASHCost costing framework as a guide. WASHCost is a well-accepted methodology in the WASH sector that provides a complete accounting not only of the upfront capital expenses, but also expenditures on capital, maintenance and local support (e.g., community water committees and local governments).

- **Latrines, water points, and wash stations**: the study coordinator will record the costs of labor, supervision, materials and supplies, and transport of materials to the community.
- **Hygiene promotion:** we will record the costs of printed educational materials and supplies for schools, printed materials for workshops, and soap and wash station costs.
- **Salaries & Per Diems:** We will include the salaries of the hygiene cluster coordinators and the per diems given to teachers and soap-makers during their respective workshops, and also transportation costs for these individuals.
- **All Interventions**: We will also include as costs the time of community members who volunteer time for one of the interventions (e.g., helping with water point-building or latrine-building, attending workshops or trainings).
- **Maintenance:** We will make special efforts to enumerate the maintenance costs of the intervention during the second and third year of the trial. Maintenance costs will include the personnel costs of the education and sanitation cluster coordinator and health promoters, any supplies and equipment needed for maintenance of the water points or latrines, and the time of community members spent at hygiene workshops and repairing latrines or water points.
- **Fixed Costs:** Finally, we will include a portion of the fixed costs of maintaining the Carter Center office. We will report costs separately for each intervention, as costs per community.

As in previous studies, costing data will be collected at the time the costs are incurred, using standardized forms. We will not include certain protocol-induced costs in the intervention costs. For example, we will not include the costs of the study coordinator, census, or monitoring visits, which are costs imposed by the trial protocol, and would not exist outside the trial. We will, however, keep track of these costs, since they may be useful for secondary analyses.

11.1.2 ***Decision Model-based CEA***

We will use WASHCost's life-cycle costing approach to model the long-term costs of the WASH intervention. We will estimate future costs based on maintenance costs recorded from the second and third year of the trial and using assumptions about the lifetime of the hardware (water points, latrines, and wash stations) based on interviews with local service providers. Future costs will be discounted to adjust them to their present value.

11.2 Assessment of effectiveness

Effectiveness outcomes are discussed elsewhere in this Manual of Procedures, but include:

- Ocular chlamydia (from monitoring visits)
- Mortality (from census)
- Stunting (from anthropometric monitoring)
- All-cause and cause-specific health clinic visits (from clinic based case finding)

12.TREATMENT

12.1 Mass albendazole distribution

The clusters were initially dewormed with a single mass albendazole distribution to all pre-school aged individuals who are 1 year or older (200mg for children aged 12-23 months and 400mg for individuals 24 months and older). Another albendazole treatment is not scheduled.

12.2 Mass azithromycin distribution

12.2.1 ***Mass azithromycin distribution during SWIFT I***

The WagHemra zone received 8+ annual rounds of mass azithromycin before the start of the trial, with the most recent treatment occuring 6 months before the baseline visit. No mass azithromycin distributions are planned during SWIFT I.

If the average prevalence of ocular chlamydia in 0-5-year-old children is too high, we will conduct a mass azithromycin treatment immediately after the next scheduled annual monitoring visit. No threshold is set, but consideration will be given to prevalence above 20%.

During the mass treatment, all individuals aged 6 months and up will be offered a single dose of oral azithromycin, 20mg/kg for children using height-based dosing and 1g for adults. [64] Those under 6 months of age, pregnant, or macrolide-allergic would be offered 2 tubes of topical tetracycline ointment, to be used twice daily.

All age groups and sexes are eligible to receive azithromycin as per each study arm outlined above, except those contraindicated by Federal Ministry of Health, which currently are:

- Those self-reported as pregnant
- Children under six months old
- Those known to be allergic to azithromycin or macrolides such as erythromycin

These three exceptions will be treated with topical tetracycline eye ointment.

12.3 Adherence to Treatment

Adherence to azithromycin treatment will be essentially 100% of those treated since administration of the single dose of antibiotic is directly observed by the distribution team.

12.4 Adverse Outcomes and Patient Death

More than 450 million doses of oral azithromycin have now been distributed for trachoma, and reports of serious side effects are essentially non-existent. This may be due in part to minimal surveillance. It also may be due to the fact these are extremely rare with a single dose of azithromycin. In fact, where carefully monitored, there were actually fewer GI side effects after taking azithromycin. We will create a network to identify any possible post-treatment serious adverse effects.

Azithromycin is generally well-tolerated. The most common side effects of azithromycin and erythromycin are diarrhea or loose stools, nausea, abdominal pain, and vomiting, each of which may occur in fewer than one in twenty persons who receive azithromycin. Rarer side effects include abnormal liver function tests, allergic reactions, and nervousness. Diarrhea due to *Clostridium difficile* has been rarely reported.

12.4.1 ***Adverse Outcomes***

Non-serious side effects are not uncommon, and serious side effects are possible. The inability of even frequent mass azithromycin distributions to bring about elimination in areas with highly prevalent trachoma suggests that other, non-antibiotic measures may be needed.

The **adverse reactions** that may occur after taking azithromycin will be explained to individuals prior to enrollment in this study. In the event of an adverse outcome, the patient will no longer be enrolled in the study and an alternative treatment for trachoma (e.g. tetracycline ointment) will be administered if the patient needs to continue treatment. If a patient experiences a serious adverse outcome, they will be advised to alert the cluster leader, who will then inform the health care representative. This person will in turn inform The Carter Center study coordinator, who will contact the Co-Investigator in Addis Ababa. If, for any reason, they will need further eye care, they will be referred to the nearest health center for examination and treatment, and the most appropriate action will be taken to provide immediate care.

All individuals who have been given azithromycin will be told to immediately communicate side-effects to local health extension workers, who will relay the message to representatives of The Carter Center program, which will ensure that appropriate medical care will be provided, and that the frequency and severity of adverse events can be assessed. In addition, all adverse events will be recorded and monitored for each individual and reports on adverse events will be made to the DSMC.

12.4.2 ***Patient Death***

The infant mortality rate is quite high in this area of Ethiopia. All deaths since the first census will be carefully recorded during the study. Since the major causes of infant mortality in the area are diarrhea, respiratory infections, and malaria, it is possible that receiving Azithromycin treatment may actually have a positive effect.

All death records (if available) will be maintained by the study coordinator at The Carter Center Ethiopia. The incidence of mortality for each study arm will be made available to the DSMC by the biostatistician.

12.5 Study Medication Description

12.5.1 ***Zithromax***

Zithromax® is supplied for oral administration as film-coated, modified capsular shaped tablets containing azithromycin dehydrate equivalent to either 250mg or 500mg azithromycin and the following inactive ingredients: dibasic calcium phosphate anhydrous, pregelatinized starch, sodium

croscarmellose, magnesium stearate, sodium lauryl sulfate, hydroxypropyl methylcellulose, lactose, titanium dioxide, triacetin and D&C red #30 aluminum lake.

Zithromax® for oral suspension is supplied in bottles containing azithromycin dehydrate powder equivalent to 300mg, 600mg, 900mg, or 1200mg azithromycin per bottle and the following inactive ingredients: sucrose; sodium phosphate, tribasic, anhydrous; hydroxypropyl cellulose; xanthan gum; FD&C Red #40; and spray dried artificial cherry, crème de vanilla and banana flavors. After constitution, each 5mL of suspension contains 100mg or 200mg of azithromycin.

12.5.2 ***Albendazole***

Albendazole is available in generic form in Ethiopia as a chewable fruit-flavored 400mg tablet.

12.6 Dosage Information

12.6.1 ***Azithromycin***

Azithromycin will be administered as a single dose, in tablet form for adults and in oral suspension form for children. Dosing will be as per the WHO recommendations for treatment of active trachoma:

1) Single dose of one gram of azithromycin for adults
2) Single dose of 20mg/kg in children (up to the maximum adult dose of 1g)
3) Height-based dosing of children will be acceptable, as per The Carter Center's program—note that this is supported by the WHO PBD group.

Individuals who are either under the age of 6 months, pregnant, or allergic to macrolides/azalides will be treated with 1% tetracycline eye ointment to be applied twice daily to both eyes for a 6-week period.

12.6.2 ***Albendazole***

Albendazole will be administered to all individuals aged 1 year and up. Children aged 12-23 months will receive half a 400mg tablet (i.e., 200mg) and all other individuals will receive a single 400mg tablet, as per WHO recommendations. Individuals with a known allergy will not receive deworming treatment.

12.7 Alternate Therapies

12.7.1 ***Alternates to Zithromax: Tetracycline Ophthalmic Ointment***

Tetracycline ophthalmic ointment (1%) is the current standard treatment in Ethiopia for ocular trachoma, and will be distributed to study patients who are not eligible to receive azithromycin.

12.8 Treatment/Monitoring Schedule

As discussed, if the average prevalence of ocular chlamydia in 0-5-year-old children is greater than 20% in the study, we may conduct a mass azithromycin treatment immediately after the next scheduled annual monitoring visit.

12.9 Medication Procurement/Donation

Pfizer, Inc. will provide the donation of Zithromax® (azithromycin), which will be shipped directly to Ethiopia and received by a representative of the Ethiopian Ministry of Health, who will manage the customs process and transport the medication from the port to a storage site. The exemption of duties and taxes will be settled by the Ethiopian Customs Authorities and the Ethiopian Ministry of Health.

12.10 Study Medication Storage and Accountability

Zithromax® tablets will be stored between 15° to 30°C (59° to 86°F), as recommended by Pfizer. A record of the exact number of tablets distributed and quantity of oral suspension dispensed will be kept by The Carter Center treatment distribution team.

Albendazole tablets will be stored between 20° and -25°C, as recommended by the manufacturer.

12.11 Medication Quality Control

Study medication will be stored in The Carter Center project office prior to use. The hygiene cluster coordinator and the study coordinator will regularly check and record the study medication expiry dates. The expiration dates on the medication containers will be strictly monitored and all expired study medicine will be discarded appropriately.

12.12 Checking Antibiotic Coverage

The application allows the users to monitor antibiotic treatment coverage, since treatment is recorded in the application. The application supplies all individuals needing treatment in the app, and treatment is then entered into this page. Treatment coverage can be viewed on Salesforce.com in real time.

The study coordinator will also monitor treatment coverage by visiting the clusters after treatment has occurred and arbitrarily choosing individuals in the cluster to see if they were treated as marked.

13.PROTECTION OF HUMAN SUBJECTS

13.1 Internal Review Board Approval

13.1.1 ***UCSF Committee on Human Research***

The University of California, San Francisco, Committee on Human Research will annually review study protocol for ethical approval.

13.1.2 ***The Amhara Region, the Ethiopian National Research Ethics Review Committee (NRERC/MoST), and the Ethiopian FDA (formerly FMHACA)***

The study protocol will be reviewed and granted ethical approval by the above three organizations before any study activities begin.

13.1.3 ***Emory University Institutional Review Board***

The Emory University Institutional Review Board will annually review study protocol for ethical approval.

13.2 Informed Consent

The head of each kebele will be asked for permission to include the cluster in the study. Additionally, the study will be discussed with all adult family members in the cluster by the participating Carter Center staff members who speak Amharic or the other local language.

At each collection visit, parents/guardians of study participants who are 0-6 years of age will be informed about the possible risks and benefits examination, swabbing, photography, and treatment (if applicable), and asked to give a verbal consent. Young adults and children below 18 years of age, who cannot give consent by law, will be included in the study only following the receipt of verbal informed consent from a parent or guardian. Verbal assent will be obtained by any child over the age of 7. If, at any time, a parent or guardian elects to withdraw themselves or a family member from the study, it will be made clear that they will be offered the same medical treatment outside the study.

13.3 Adequacy of Protection Against Risk

There are several layers of procedures to help minimize study-associated risk to participants.

13.3.1 ***Conjunctival swabbing***

There are minimal risks to the subject who receives conjunctival swabbing for chlamydia. We are aware of no reported complications from this procedure, although a small amount of conjunctival bleeding can occur and a corneal abrasion is possible. Any adverse effects will be treated by the examiners. Ocular examinations will be offered to everyone, even if they choose not to participate in the study. Appropriate ophthalmic care or referral will be provided for any conditions detected during these examinations, regardless of participation in the study.

13.3.2 ***Nasopharyngeal swabbing***

Nasopharyngeal swabbing causes some temporary discomfort but it involves minimal risks without further complications. Any adverse effects, such as nose-bleeds, will be treated immediately by the examiners. Other health care will be provided at no cost to the study participant if necessary, to address a study-related adverse health event.

13.3.3 ***Clinical photography***

Clinical photography of the conjunctiva causes no damage to the eye, is well tolerated by children, and is a standard clinical procedure at UCSF.

13.3.4 ***Treatment***

If treatment is necessary, the risk of antibiotic treatment will be minimized by treating only those who fit in the approved age and inclusion category, as well as by regularly scheduled follow-up examinations by a trained trachoma grader. Should the antibiotic be ineffective to an individual, the study medication will be discontinued for them. In the event of any adverse effects, appropriate medical care will be provided by the local health center.

13.4 Inclusion of Pregnant Women and Children

All participants, regardless of gender, will be accepted. We will obtain informed consent from all study participants prior to entering the study.

If treatment is necessary, pregnant women will be excluded from receiving oral azithromycin, and will be offered topical tetracycline eye ointment in its place.

13.5 Compensation to Participants

There is no cost to the participant and there is no reimbursement for overall participation in this study. Each participant will receive free ophthalmic examinations during the course of the study.

14.DATA AND SAFETY MONITORING COMMITTEE CHARTER

The Charter will define the primary responsibilities of the DSMC, its relationship with other trial components, its membership, and the purpose and timing of its meetings. The Charter will also provide the procedures for ensuring confidentiality and communication, statistical monitoring guidelines to be implemented by the DSMC, and an outline of the content of the Open and Closed Reports that will be provided to the DSMC.

14.1 Primary Responsibilities of the DSMC

The DSMC will be responsible for safeguarding the interests of trial participants, assessing the safety and efficacy of the interventions during the trial, and monitoring the overall conduct of the trial. The DSMC will provide recommendations about stopping or continuing the trial. To contribute to the integrity of the trial, the DSMC may also formulate recommendations relating to the selection/ recruitment/ retention of participants, to protocol-specified regimens, and the procedures for data management and quality control.

A chief responsibility of the DSMC will be to review data on ocular chlamydia prevalence and make recommendations regarding the need for mass azithromycin treatments. The DSMC will make a decision about thresholds for initiating treatment at each meeting. The DSMC purposefully did not set a threshold to trigger re-treatment, but rather a threshold triggering a discussion about whether antibiotic treatment was needed. This threshold is 20% prevalence of ocular chlamydia among the 0-5-year-old age group.

The DSMC will be advisory to the trial leadership group, hereafter referred to as the Executive Committee (EC). The EC will be responsible for promptly reviewing the DSMC recommendations and determining, whether to continue or terminate the trial, and to determine whether amendments to the protocol are required. If needed, the DSMC may seek the advice of a content expert outside of the committee.

14.2 DSMC Membership

The DSMC is an independent multidisciplinary group consisting of epidemiologists, biostatisticians, bioethicists, and clinicians that collectively has experience in the management of infectious diseases and in the conduct and monitoring of randomized clinical trials including subsaharan Africa.

14.3 Conflicts of Interest

The DSMC membership has been restricted to individuals free of apparent conflicts of interest. The source of these conflicts may be financial, scientific, or regulatory. Thus, neither study investigators nor individuals employed by the sponsor, nor individuals who might have regulatory responsibilities for the trial products, are members of the DSMC.

The DSMC members will disclose to fellow members any consulting agreements or financial interests they have with the sponsor of the trial, with the contract research organizations (CRO), or with other

sponsors having products that are being evaluated or that are competitive with those in the trial. The DSMC will be responsible for deciding whether these consulting agreements or financial interests materially impact their objectivity.

The DSMC members will be responsible for advising fellow members of any changes in any of the membership requirements that occur during the course of the trial. It may be appropriate for DSMC members who develop significant conflicts of interest resign from the DSMC.

DSMC membership is to be for the full duration of the trial. If any members leave the DSMC, the EC, in consultation with the DSMC, will promptly appoint a replacement.

14.4 Timing and Purpose of the DSMC Meetings

14.4.1 ***Organizational Meeting***

The initial meeting of the DSMC was held 16 September 2014. The committee provided an advisory review of scientific and ethical issues relating to study design and discussed the standard operating procedures, as well as the format and content of the Open and Closed Reports that will be used to present trial results.

The Organizational Meeting was attended by all DSMC members, lead trial investigators, and the trial biostatistician. The DSMC reviewed drafts of the trial protocol, the Statistical Analysis Plan, and the DSMC Charter. At subsequent meetings, committee members will receive Data Reports.

14.4.2 ***Future meetings***

Future DSMC meetings will be held on a yearly basis, with an option for more frequent phone meetings or email updates at the discretion of the DSMC and/or principal investigator.

14.5 Procedures to Ensure Confidentiality and Proper Communication

To enhance the integrity and credibility of the trial, procedures will be implemented to ensure the DSMC has access to all emerging information from the trial regarding comparative results of efficacy and safety, aggregated by treatment arm.

14.5.1 ***Closed Sessions***

Sessions involving only DSMC members and, where appropriate, those unmasked trial investigators (on the Data Coordinating Committee) who generate the Closed Reports (called Closed Sessions) will be held to allow discussion of confidential data from the trial, including information about the relative efficacy and safety of interventions.

At a final Closed Session, the DSMC will develop a consensus on its list of recommendations, including that relating to whether the trial should continue.

14.5.2 ***Open Session***

In order for the DSMC to have access to information provided, by study investigators, or members of regulatory authorities, a joint session between these individuals and DSMC members will be held between the Closed Sessions.

14.5.3 ***Progress Reports***

For each DSMC meeting, a report will be provided. The report will include data on recruitment and baseline characteristics, exam coverage, exam results, pooled data on eligibility violations, adverse events, and completeness of follow-up and compliance. The data analyst will prepare this report.

The report should provide information that is accurate, with follow-up that is complete to within two months of the date of the DSMC meeting. The Reports should be provided to DSMC members approximately three days prior to the date of the meeting.

14.6 Minutes of the DSMC Meeting

The research team will prepare minutes for the open portion of the meeting, including the DSMC's recommendations.

14.7 Recommendations to the Executive Committee (EC)

At each meeting of the DSMC during the trial, the committee will make a recommendation to the Executive Committee to continue or terminate. This recommendation will be based primarily on safety and efficacy considerations and will be guided by statistical monitoring guidelines defined in this Charter.

Recommendations to amend the protocol or conduct of the study made by the DSMC will be considered and accepted or rejected by the EC. The EC will be responsible for deciding whether to continue or to stop the trial based on the DSMC recommendations.

The DSMC will be notified of all changes to the protocol or to study conduct. The DSMC concurrence will be sought on all substantive recommendations or changes to the protocol or study conduct prior to implementation.

The EC may communicate information in the Open Report to the sponsor and may inform them of the DSMC recommended alterations to study conduct or early trial termination in instances in which the EC has reached a final decision agreeing with the recommendation. The EC will maintain confidentiality of all information it receives other than that contained in the Open Reports until after the trial is completed or until a decision for early termination has been made.

14.8 DSMC Contact Information

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15. DATA COLLECTION, MANAGEMENT, AND QUALITY ASSURANCE

Similar to our previous studies, all study personnel who assess outcomes will attend an intensive, 2-day training session prior to the annual monitoring visits. Study personnel will collect all written data from the census and monitoring visits using tablet computers. We successfully pilot tested this electronic data capture system in Ethiopia in 2011 and are currently using it for a separate clinical trial in Niger.

15.1 Data Collection Tools

15.1.1 ***Tablet Computers***

The Proctor Foundation will provide training on operating tablet computers to all data collectors. In our experience, a tablet computer typically retains its charge for a full day's activities, though each team will also have a backup battery pack. Tablets and batteries will be charged overnight in a safe, secure place.

15.2 Mobile Application

A custom mobile application will be used for all census and monitoring visits. This software assigns a unique identification number to each study participant enumerated during the census. The software integrates the monitoring visits with the census information by linking to this unique identifier. We will provide training to use this mobile application for all study activities.

15.3 Data Transfer

We will upload the data from the tablet computers directly to Salesforce.com.

15.4 Data Quality

Electronic data capture will ensure high quality data since it removes the possibility for transcription error and allows for validation rules in data entry fields.

15.5 Data Consistency and Validity

Through range checks, the software ensures to a large extent that there are no inconsistencies or invalid data. The software will create an error file with relevant data such as the form identification, field names and data. Data consistency and errors will also be monitored.

15.6 Data Collection

15.6.1 ***Census Administration***

Basic protocols for census taking are available in the Amharic language.

15.6.2 ***Completing the Census***

The census team will visit each cluster and inform the village leader to alert each head of household in the village. The census form will contain the following:

- Name
- Gender

- Age of each household member
- GPS coordinates for each household
- Health facilities, water points, and schools utilized
- Presence or absence of latrine and latrine-use
- Presence or absence of hand-washing facility near latrine
- Household water-use and hygiene behaviors

Household numbers and individual numbers for further identification of individuals within each household will be automatically assigned.

15.7 Examination, Swabbing, and Anthropometry

15.7.1 ***Registration***

Registration is completed by the exam team, and the registration lists contains the full spelling of each individual's name, age, gender and household number, taken directly from the census.

15.7.2 ***Swabbing***

Contamination of eye swabs can occur in trachoma studies; therefore, we will collect negative control conjunctival swab on a 10% random sample during all monitoring visits to assess the possibility of contamination.

15.7.3 ***Anthropometry***

Height, weight, and MUAC will be entered in triplicate for each study participant. The presence or absence of kwashiorkor and marasmus will be noted for each participant. This data will be entered into the app, which employs data validation.

15.8 Costing

Data will be collected on standardized forms. The Proctor Foundation study coordinator and Carter Center study coordinator will be responsible for training the hygiene cluster coordinator and driver in filling out the costing forms. We will use paper cost forms, with data double-entered into a database by Carter Center personnel in Addis Ababa. Computers will be automatically backed up once daily.

15.9 Data Security and Storage

Databases at the central site will be stored on an encrypted server in a temperature-controlled locked room at the Proctor site, and off-site backups will be maintained. Backup encryption keys will be maintained off-site in a secure vault. Database procedures will include full transaction logs. The DSMC can make requests to have access to the data at any point during the course of the study.

15.10 Data Analysis Estimation of Disease Prevalence

Computerized randomization is utilized to prepare samples for pooling. PCR pooling will be conducted stratified by age stratum and study community. In other words, in any given community, swabs from 0-5-year-olds will be pooled together, swabs from 6-9 year-olds (WUHA), 8-12 year-olds (TAITU) , and ≥

10-year-olds (WUHA) will be pooled by age group. All age strata will be pooled in pools of 5. We will automatically unpool any positive pools from the 0-5-year age group and results tabulated by cluster. For the 6-9-year-old, 8-12-year-old, ≥ 10-year-old age stratum, we will calculate the prevalence using the age group pools. Specifically, we use maximum likelihood estimation to determine what prevalence in the population (by the 3 older age strata, by cluster) has the highest likelihood of resulting in the observed pooled results (the likelihood is a relatively simple function of the number of positive and negative 5-pools, with appropriate combinatorics).

15.11 Sample Organization and Storage

Samples will be stored at the Amhara Public Health Institute in -20° freezers. APHI has a protocol to monitor the temperature of the freezers and switch to generator power when needed. The samples are automatically linked to the person-data via the Salesforce.com database and the application that was built for this study.

16. STATISTICAL ANALYSES

Statistical analyses and sample size calculations are detailed in a separate Statistical Analysis Plan.

17.APPENDICES

17.1 Swift-STH Supplement

Rationale

Water, sanitation, and hygiene (WASH) interventions are thought to be important for reducing transmission of several neglected tropical diseases, including trachoma and soil transmitted helminths. However, very few clinical trials have been conducted to assess the relative benefits of WASH interventions. Given the expense of implementing a WASH package that includes water point and latrine construction, it is important that clinical trials be conducted to assess the efficacy of WASH for neglected tropical diseases.

The Francis I. Proctor Foundation at the University of California, San Francisco was recently awarded a 5-year grant by the National Eye Institute to assess the efficacy of a comprehensive WASH package for trachoma (grant number U10EY023939, PI Keenan). Although the grant submission focused on trachoma, the investigators of the trial realize that a WASH package should have many benefits besides reduction of trachoma and intend to study additional outcomes. Specifically, we wish to collect stool samples to assess for soil-transmitted helminths, Necator americanus, Ancylostoma duodenale, Trichuris trichiura, Ascaris lumbricoides, and Strongyloides stercoralis and other intestinal pathogens, as well as dried blood spots to assess serologic markers of trachoma, helminthic infections, and intestinal bacterial or protozoal infections. Knowledge of these other potential health benefits provided by a WASH package will help us to better assess the ultimate cost-effectiveness of implementing WASH in sub-Saharan Africa.

Study Design

General study design: We are organizing a cluster-randomized trial of 40 study clusters in Ethiopia in which half receive a comprehensive WASH package and the other half do not receive any interventions until the end of the trial. We will perform annual monitoring of study clusters for three years. Our primary outcome is the prevalence of ocular chlamydia in 0-5-year-old children at 36 months. We will also monitor for several secondary outcomes, including facial cleanliness, anthropometry, nasopharyngeal pneumococcus, soil-transmitted helminths, and heath post visits for infectious illnesses. We hypothesize that the WASH clusters will have a lower prevalence of these infectious diseases than the control clusters.

Study area: The study will take place in 3 woredas of the WagHimra zone, Amhara Region, Ethiopia. Despite 8 years of annual mass azithromycin treatments, WagHimra still has hyperendemic trachoma, with recent surveys showing clinically active trachoma in approximately 60% of 1-9-year-old children.

Randomization unit: We plan to use school catchment areas as randomization units. We chose this randomization unit because infectious diseases are likely transmitted at schools. In addition, we wish

to provide hygiene promotion activities through schools and want to avoid contamination between clusters of the trial.

Population of interest: Although we randomize school catchment areas, outcomes will be assessed only in a single cluster per school district. We will perform a geohydrologic survey and work with local water boards to identify a potential location for water point construction in each school catchment area, and will monitor all outcomes in the 1.5-kilometer radius surrounding the potential water point. Note that we will monitor the households around the potential water site in both the intervention and control study arms, but only the intervention communities will have a water point constructed.

Formative research: We will promote several key hygiene messages throughout the trial in an attempt to improve adherence to facial hygiene in the intervention clusters. We intend to focus our hygiene promotion on messages encouraging habit formation (e.g., "wash your face every morning"). We will fine-tune messaging and try to identify barriers to improving hygiene behaviors through the use of focus group discussions at the beginning of the study.

Census: We will perform a population census of all selected study clusters each year of the trial using an electronic data capture system developed for a Gates-funded trial of mass azithromycin for childhood mortality.

Mass drug administration: All communities will receive a single mass azithromycin distribution and a single mass albendazole distribution at the beginning of the study. After this time, no further mass distributions are planned.

WASH Interventions: We will hire one local "hygiene cluster coordinator(s)" to assist the study coordinator with all aspects of the WASH package and to ensure high uptake of WASH interventions in all study clusters.

- **Water:** we will work with local nongovernmental organizations to construct a water point (spring development, hand dug well, or shallow borehole) in each intervention community. Study staff will assist with the formation of a local water committee to handle the finances for and maintenance of the water point.
- **Sanitation:** we will promote construction of one usable latrine per household. We will work within the regulations of the Ethiopian government for this activity, which currently mandate construction of simple pit latrines.
- **Hygiene:** Hygiene promotion will focus on habit formation surrounding face washing, hand washing, and latrine use. We do not plan on transferring knowledge about infectious diseases but rather emphasizing simple habits that will improve hygiene behaviors. In order to help habit formation, we will implement 2 "hardware" components: first, we will provide a wash station at each household, and second, we will provide soap for the duration of the trial. We will spread our hygiene messages in several forums, which will be based on the results of the focus group discussions. The main person who will do this work will be the hygiene promotion worker. Additionally, from our experience with focus groups this past year in Ethiopia, most people thought

government-appointed health extension workers and local priests would be the best people to discuss these hygiene improvements. We will also print posters and pamphlets that can be distributed at any hygiene workshops, and will work with schools to implement a hygiene workshop for all school children.

WASH process indicators: We will use spot checks, an annual WASH survey, and structured observations to assess whether the WASH interventions were implemented as planned. We will report specific metrics of the uptake of the intervention, based on random spot-checks of intervention study clusters, direct observation of latrines and wash stations during the census, and observation of the newly constructed water points. We will perform this monitoring each year and will take measures to improve the uptake if necessary.

Outcomes: We will perform monitoring of all 0-5-year-old children in each cluster, as well as a random sample of 30 children aged 6-9-years and a random sample of 30 individuals aged ≥10 years. In addition, we will perform anthropometry and trachoma examination on all children who make up the cohort that was aged 0-5-years at the beginning of the study.

- **Primary outcome:** Prevalence of ocular chlamydia in 0-5-year-old children at 36 months.
- **Secondary outcomes:** We will assess several secondary outcomes, including ocular chlamydia in the remaining age groups, clinically active trachoma in all age groups, anthropometry (height and weight) in the cohort of children aged 0-5 years at baseline, nasopharyngeal pneumococcus in 0-5-year-olds, and age-stratified health post visits for diarrhea and other infections.
- **Unfunded but planned secondary outcomes:** soil-transmitted helminths in 0-9-year-olds, dried blood spots in 0-9-year-olds, microbiome of stool and nasopharynx in 0-9-year-olds.

Cost-effectiveness analysis (CEA): We will conduct a trial-based CEA over the time horizon of the trial as well as a model-based cost-effectiveness analysis that extrapolates beyond the dates of the trial. We will calculate the cost per prevented infection for the trial-based analysis, and the cost per DALY averted for the model-based CEA.

17.2 Rectal swabs protocol

STUDY DESIGN

Specific Aim 4a: To determine whether albendazole and oral azithromycin distribution alters the intestinal microbiome.

> **Hypothesis 4a.** We hypothesize that 1 week after distribution of oral albendazole, the intestinal microbiome of treated children will be significantly different from that of untreated children and also different from that of children treated with oral azithromycin.

Specific Aim 4b: To determine the validity of using rectal swabs versus bulk stool samples for measuring the intestinal microbiome and soil transmitted helminths (STHs).

> **Hypothesis 4b.** We hypothesize that measuring microbiome and detecting STH using rectal swabs will be equivalent to using bulk stool samples.

Intervention: In communities selected for the albendazole sub study (Specific Aim 4), children will be randomized in a factorial design to oral albendazole treatment on day 0 versus albendazole treatment on day 7, and to azithromycin treatment on day 0 versus azithromycin treatment on day 7. Note that all children will receive both a single dose of azithromycin and a single dose of albendazole, but the only difference is that the doses will be spaced 1 week apart.

Primary outcome: Intestinal microbiome from stool sample, using 16S rRNA deep sequencing and/or next generation sequencing:

- We will perform an intention-to-treat analysis. The primary analysis for the albendazole and oral azithromycin arms will be performed in the population-based sample of children aged 0-5 years at each study visit using community-level data. We will perform 16S rRNA Gene Deep Sequencing on the bulk stool samples and compare the bacterial diversity between the arms using the Simpson's diversity index and visualized using multidimensional scaling (MDS) and principal coordinate analysis (PCoA) in the R environment. (Parks DH, Beiko RG. Identifying biologically relevant differences between metagenomic communities. *Bioinformatics*. 2010;26:715-721.)

Secondary Outcome 1: STH PCR in stool and rectal swab

- We are already approved to perform PCR analysis for STH on bulk stool samples. We will also perform PCR on rectal swabs. Using bulk stool samples as the gold standard, we will assess the sensitivity and specificity of detecting STH using rectal swabs with logistic mixed-effects treating community as a random effect.

Secondary Outcome 2: Intestinal microbiome in stool sample and rectal swab, using 16S rRNA deep sequencing and/or next generation sequencing

- Using the same 16S rRNA deep sequencing, we will assess the sensitivity and specificity for detecting various enteric pathogenic bacteria from rectal swabs compared to the gold standard method (bulk stool samples). We will use logistic mixed-effects treating community as a random effect.

PROCEDURES

Stool Assessment: We will collect both bulk stool specimens and rectal swabs. These samples will be used for 4 techniques. SAF ether-concentration and PCR of stool STH techniques are already approved. With this amendment, we will also collect samples for PCR for rectal swabs and 16S RNA/next generation deep sequencing. The agreement between bulk stool samples and the rectal swabs for measuring these 3 outcomes will be compared.

General stool sample collection procedures: We have been previously approved to collect stool samples from 0-9-year-old children for SAF and PCR testing.

General rectal swab procedures: We will collect a rectal swab from a random sample of children 0-5. Swabs will be collected in the routine fashion. For most children, the parent will position the child with the child's stomach on the parent's lap, and the child's legs hanging off the lap, oriented toward the ground. For small children, the parent will lie the child's back on the parent's lap, and hold the legs up in the air. The skin around the child's anus will be cleaned using a new sterile disinfectant wipe designed and sold for babies. Then, using a sterile fecal swab collection kit, the examiner will insert the tip of the cotton swab 1-3 cm into the child's anus until it reaches fecal material, and gently twist the swab as it is removed. The swab will then be placed in a tube with preservative.

Soil-Transmitted Helminths: As previously approved, we will use 2 methods to detect STH in the bulk stool samples- 1) preserving stool in SAF to count eggs and parasites; and 2) storing stool in 5% potassium dichromate solution for PCR processing. A 3-gram sample of stool will be stored in sodium acetate-acetic acid-formalin (SAF) at room temperature in the field, and then transported to the Amhara Public Health Institute for processing. As was also previously approved, the PCR samples will be transported to UCSF and Smith College in the United States where trained laboratory personnel will perform novel PCR analysis and determine the presence of the major soil transmitted helminths (*Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm), as well as the density of each. Rectal swabs will be transported with the bulk PCR stool samples and will undergo the same PCR assay.

Deep sequencing: Stool samples and rectal swabs taken for 16S RNA will be transported to UCSF and processed with deep sequencing methods. Methods include 16S ribosomal RNA sequencing for bacterial pathogens, as well as next generation sequencing. These methods will provide evidence on diarrheal pathogens as well as the enteric microbiome.

Location of sample processing: Please note that both rectal swab sample processing using PRC assay and stool and rectal swab analysis using 16S RNA/deep sequencing are novel methods of stool sample analysis, which are still being validated. We are hoping that this study will provide valuable data to help in the validation of these techniques. Because these testing methods are still being validated, they are not ideal candidates for processing in Ethiopia. However, it is important to note that we are committed to capacity building, and are processing chlamydia samples, nasopharyngeal samples, and SAF stool samples at the APHI.

RISKS AND BENEFITS

Risks and discomforts:

Stool sample: Stool samples have been collected in this setting before, with essentially no risk to participants.
Rectal swab: Rectal swabs are a simple procedure with very minimal risk.

Medication risks: Co-administration of azithromycin and albendazole has been shown to be safe in multiple studies (Am J Trop Med Hyg 2007; 76:1153; PLoS Negl Trop Dis 2013; 7:e2221).

Steps to minimize risks:
Stool samples: Examiners will encourage children and parents to wash hands after providing a stool sample.
Rectal swabs: Examiners will be thoroughly trained to make children feel comfortable during the rectal swab. We will take children to a private location with their caregiver to minimize discomfort.

Benefits to society
The results of the trial will provide evidence about the importance of WASH for trachoma, nasopharyngeal pneumococcus, soil-transmitted helminths, and growth. The results will help public health planners know how to prioritize the WASH interventions when considering ways to combat neglected tropical diseases, and therefore, this knowledge will benefit society.

Explanation
The risks associated with these assessments are very minor, and the results from the tests will benefit society.

18.REFERENCES

1. West ES, Munoz B, Mkocha H, et al. Mass treatment and the effect on the load of Chlamydia trachomatis infection in a trachoma-hyperendemic community. Invest Ophthalmol Vis Sci 2005;46:83-7.
2. Burton MJ, Holland MJ, Makalo P, et al. Re-emergence of Chlamydia trachomatis infection after mass antibiotic treatment of a trachoma-endemic Gambian community: a longitudinal study. Lancet 2005;365:1321-8.
3. Solomon AW, Holland MJ, Alexander ND, et al. Mass treatment with single-dose azithromycin for trachoma. N Engl J Med 2004;351:1962-71.
4. Taylor HR, Rapoza PA, West S, et al. The epidemiology of infection in trachoma. Invest Ophthalmol Vis Sci 1989;30:1823-33.
5. West SK, Munoz B, Mkocha H, et al. Infection with Chlamydia trachomatis after mass treatment of a trachoma hyperendemic community in Tanzania: a longitudinal study. Lancet 2005;366:1296-300.
6. Skalet AH, Cevallos V, Ayele B, et al. Antibiotic selection pressure and macrolide resistance in nasopharyngeal Streptococcus pneumoniae: a cluster-randomized clinical trial. PLoS Med 2010;7:e1000377.
7. Haug S, Lakew T, Habtemariam G, et al. The decline of pneumococcal resistance after cessation of mass antibiotic distributions for trachoma. Clin Infect Dis 2010;51:571-4.
8. Ayele B, Aemere A, Gebre T, et al. Reliability of measurements performed by community-drawn anthropometrists from rural ethiopia. PloS one 2012;7:e30345.
9. Pascolini D, Mariotti SP. Global estimates of visual impairment: 2010. The British journal of ophthalmology 2012;96:614-8.
10. Mariotti SP, Pascolini D, Rose-Nussbaumer J. Trachoma: global magnitude of a preventable cause of blindness. Br J Ophthalmol 2009;93:563-8.
11. Vision Research: Needs, Gaps, and Opportunities. 2012. (Accessed January 2, 2013, at http://www.nei.nih.gov/strategicplanning/pdf/VisionResearch2012.pdf.)
12. Mariotti SP, Pararajasegaram R, Resnikoff S. Trachoma: looking forward to Global Elimination of Trachoma by 2020 (GET 2020). The American journal of tropical medicine and hygiene 2003;69:33-5.
13. Meeting of the International Task Force for Disease Eradication - October 2010. Releve epidemiologique hebdomadaire / Section d'hygiene du Secretariat de la Societe des Nations = Weekly epidemiological record / Health Section of the Secretariat of the League of Nations 2011;86:53-9.
14. House JI, Ayele B, Porco TC, et al. Assessment of herd protection against trachoma due to repeated mass antibiotic distributions: a cluster-randomised trial. Lancet 2009;373:1111-8.
15. Lakew T, House J, Hong KC, et al. Reduction and return of infectious trachoma in severely affected communities in Ethiopia. PLoS Negl Trop Dis 2009;3:e376.
16. Gebre T, Ayele B, Zerihun M, et al. Comparison of annual versus twice-yearly mass azithromycin treatment for hyperendemic trachoma in Ethiopia: a cluster-randomised trial. Lancet 2012;379:143-51.
17. West SK, Munoz B, Mkocha H, Gaydos C, Quinn T. Trachoma and ocular Chlamydia trachomatis were not eliminated three years after two rounds of mass treatment in a trachoma hyperendemic village. Invest Ophthalmol Vis Sci 2007;48:1492-7.

18. Leach AJ, Shelby-James TM, Mayo M, et al. A prospective study of the impact of community-based azithromycin treatment of trachoma on carriage and resistance of Streptococcus pneumoniae. Clin Infect Dis 1997;24:356-62.
19. Ray WA, Murray KT, Hall K, Arbogast PG, Stein CM. Azithromycin and the risk of cardiovascular death. The New England journal of medicine 2012;366:1881-90.
20. Thygeson P. Epidemiologic Observations on Trachoma in the United States. Invest Ophthalmol 1963;2:482-9.
21. Dolin PJ, Faal H, Johnson GJ, et al. Reduction of trachoma in a sub-Saharan village in absence of a disease control programme. Lancet 1997;349:1511-2.
22. Hoechsmann A, Metcalfe N, Kanjaloti S, et al. Reduction of trachoma in the absence of antibiotic treatment: evidence from a population-based survey in Malawi. Ophthalmic Epidemiol 2001;8:145-53.
23. West S, Munoz B, Lynch M, et al. Impact of face-washing on trachoma in Kongwa, Tanzania. Lancet 1995;345:155-8.
24. Resnikoff S, Peyramaure F, Bagayogo CO, Huguet P. Health education and antibiotic therapy in trachoma control. Revue internationale du trachome et de pathologie oculaire tropicale et subtropicale et de sante publique : organe de la Ligue contre le trachome avec la collaboration de l'International Organization 1995;72:89-98, 101-10.
25. Keenan JD, See CW, Moncada J, et al. Diagnostic characteristics of tests for ocular Chlamydia after mass azithromycin distributions. Investigative ophthalmology & visual science 2012;53:235-40.
26. Atik B, Thanh TT, Luong VQ, Lagree S, Dean D. Impact of annual targeted treatment on infectious trachoma and susceptibility to reinfection. JAMA 2006;296:1488-97.
27. Abdou A, Munoz BE, Nassirou B, et al. How much is not enough? A community randomized trial of a Water and Health Education programme for Trachoma and Ocular C. trachomatis infection in Niger. Tropical medicine & international health : TM & IH 2010;15:98-104.
28. Rabiu M, Alhassan MB, Ejere HO, Evans JR. Environmental sanitary interventions for preventing active trachoma. The Cochrane database of systematic reviews 2012;2:CD004003.
29. Rabiu MM, Muhammed N, Isiyaku S. Challenges of trachoma control: an assessment of the situation in northern Nigeria. Middle East African journal of ophthalmology 2011;18:115-22.
30. Emerson P, Kollmann M, MacArthur C, Bush S, Haddad D. SAFE strategy for blinding trachoma addresses sanitation, the other half of MDG7. Lancet 2012;380:27-8.
31. Report of the sixteenth meeting of the WHO alliance for the elimination of blinding trachoma by 2020. World Health Organization, 2012. (Accessed January 2, 2013, at http://www.who.int/blindness/publications/GET16Report.pdf.)
32. Sanitation Sector Status and Gap Analysis: Ethiopia. 2009. (Accessed December 31, 2012, at http://www.wsscc.org/sites/default/files/gsf_ethiopia_sector_review.pdf.)
33. Zerihun N. Trachoma in Jimma zone, south western Ethiopia. Trop Med Int Health 1997;2:1115-21.
34. Clasen T, Fabini D, Boisson S, et al. Making sanitation count: developing and testing a device for assessing latrine use in low-income settings. Environ Sci Technol 2012;46:3295-303.
35. Curtis V, Kanki B, Cousens S, et al. Evidence of behaviour change following a hygiene promotion programme in Burkina Faso. Bull World Health Organ 2001;79:518-27.

36. Luby SP, Agboatwalla M, Painter J, Altaf A, Billhimer WL, Hoekstra RM. Effect of intensive handwashing promotion on childhood diarrhea in high-risk communities in Pakistan: a randomized controlled trial. Jama 2004;291:2547-54.
37. Ram PK, Halder AK, Granger SP, et al. Is structured observation a valid technique to measure handwashing behavior? Use of acceleration sensors embedded in soap to assess reactivity to structured observation. Am J Trop Med Hyg 2010;83:1070-6.
38. Gomez JD, Nakat AC. Community participation in water and sanitation. Water Int 2002;27:343-53.
39. Loevinsohn BP. Health education interventions in developing countries: a methodological review of published articles. Int J Epidemiol 1990;19:788-94.
40. Zhang C, Mosa AJ, Hayward AS, Matthews SA. Promoting clean hands among children in Uganda: a school-based intervention using 'tippy-taps'. Public Health 2013;127:586-9.
41. Waterkeyn J, Cairncross S. Creating demand for sanitation and hygiene through Community Health Clubs: a cost-effective intervention in two districts in Zimbabwe. Soc Sci Med 2005;61:1958-70.
42. Biran A, Tabyshalieva A, Salmorbekova Z. Formative research for hygiene promotion in Kyrgyzstan. Health Policy Plan 2005;20:213-21.
43. Waterkeyn J, Cairncross S. Creating demand for sanitation and hygiene through Community Health Clubs: a cost-effective intervention in two districts in Zimbabwe. Soc Sci Med 2005;61:1958-70.
44. McCauley AP, Lynch M, Pounds MB, West S. Changing water-use patterns in a water-poor area: lessons for a trachoma intervention project. Soc Sci Med 1990;31:1233-8.
45. Lynch M, West SK, Munoz B, Kayongoya A, Taylor HR, Mmbaga BB. Testing a participatory strategy to change hygiene behaviour: face washing in central Tanzania. Trans R Soc Trop Med Hyg 1994;88:513-7.
46. Trachoma Health Education Materials Library. (Accessed December 29, 2012, at http://www.cartercenter.org/health/trachoma_education/index.html.)
47. Tobias CR, Downes A, Eddens S, Ruiz J. Building blocks for peer success: lessons learned from a train-the-trainer program. AIDS Patient Care STDS 2012;26:53-9.
48. Thylefors B, Dawson CR, Jones BR, West SK, Taylor HR. A simple system for the assessment of trachoma and its complications. Bull World Health Organ 1987;65:477-83.
49. Roper KG, Taylor HR. Comparison of clinical and photographic assessment of trachoma. Br J Ophthalmol 2009;93:811-4.
50. West SK, Taylor HR. Reliability of photographs for grading trachoma in field studies. Br J Ophthalmol 1990;74:12-3.
51. Solomon AW, Bowman RJ, Yorston D, et al. Operational evaluation of the use of photographs for grading active trachoma. Am J Trop Med Hyg 2006;74:505-8.
52. Amza A, Kadri B, Nassirou B, et al. A cluster-randomized controlled trial evaluating the effects of mass azithromycin treatment on growth and nutrition in Niger. Am J Trop Med Hyg 2013;88:138-43.
53. Labrique AB, Christian P, Klemm RD, et al. A cluster-randomized, placebo-controlled, maternal vitamin A or beta-carotene supplementation trial in Bangladesh: design and methods. Trials 2011;12:102.
54. Glasgow RE, Vogt TM, Boles SM. Evaluating the public health impact of health promotion interventions: the RE-AIM framework. Am J Public Health 1999;89:1322-7.

55. Montgomery MA, Desai MM, Elimelech M. Assessment of latrine use and quality and association with risk of trachoma in rural Tanzania. Trans R Soc Trop Med Hyg 2010;104:283-9.
56. Stoller NE, Gebre T, Ayele B, et al. Efficacy of latrine promotion on emergence of infection with ocular Chlamydia trachomatis after mass antibiotic treatment: a cluster-randomized trial. Int Health 2011;3:75-84.
57. Weinstein MC, O'Brien B, Hornberger J, et al. Principles of good practice for decision analytic modeling in health-care evaluation: Report of the ISPOR task force on good research practices-modeling studies. Value Health 2003;6:9-17.
58. Ramsey S, Willke R, Briggs A, et al. Good research practices for cost-effectiveness analysis alongside clinical trials: The ISPOR RCT-CEA task force report. Value Health 2005;8:521-33.
59. Moller JK, Pedersen LN, Persson K. Comparison of the Abbott RealTime CT new formulation assay with two other commercial assays for detection of wild-type and new variant strains of Chlamydia trachomatis. Journal of clinical microbiology 2010;48:440-3.
60. Cheng A, Qian Q, Kirby JE. Evaluation of the Abbott RealTime CT/NG assay in comparison to the Roche Cobas Amplicor CT/NG assay. Journal of clinical microbiology 2011;49:1294-300.
61. Morre SA, van Dijk R, Meijer CJ, van den Brule AJ, Kjaer SK, Munk C. Pooling cervical swabs for detection of Chlamydia trachomatis by PCR: sensitivity, dilution, inhibition, and cost-saving aspects. Journal of clinical microbiology 2001;39:2375-6.
62. Diamant J, Benis R, Schachter J, et al. Pooling of Chlamydia laboratory tests to determine the prevalence of ocular Chlamydia trachomatis infection. Ophthalmic Epidemiol 2001;8:109-17.
63. Melese M, Alemayehu W, Lakew T, et al. Comparison of annual and biannual mass antibiotic administration for elimination of infectious trachoma. Jama 2008;299:778-84.
64. Basilion EV, Kilima PM, Mecaskey JW. Simplification and improvement of height-based azithromycin treatment for paediatric trachoma. Trans R Soc Trop Med Hyg 2005;99:6-12.

19. SWIFT REVISIONS HISTORY

9 October 2015 – Version 2.4 includes the following updates since Version 2.3 (5 Sept 2015):

1. Addition of second year for TAITU trial
2. Addition of delayed treatment arm to TAITU trial
3. Addition of Hypothesis 2A comparing targeted to delayed antibiotic treatment
4. Addition of TAITU name (Targeted Antibiotic Intervention for Trachoma in Under-5s) for targeted antibiotic study
5. Addition of mass albendazole distribution to WUHA intervention
6. Addition of plan to develop and implement grade specific lesson plans and WASH Book
7. Specification of Catholic Relief Services as the well implementing organization
8. Addition of information about the water feasibility study and randomization plan
9. Change from initial plan for implementation of sanitation hardware (latrine slab purchase and installation) to the current plan to support slab producer groups
10. Change in implementing organization for focus group discussions from Proctor Foundation to Carter Center
11. Change in type of wash station being implemented from tippy tap to jerry can hand washing station with tap
12. Clarification of active surveillance tests to be completed for each age strata
13. Removal of plans for other serological testing methods outside of dried blood spots
14. Updates to timeline of initial study activities and timeline of interventions and monitoring visits
15. Removal of the database specialist position at APHI. The laboratory technician and microbiologist will fulfill the database duties.
16. Change in name of passive surveillance to “clinic-based case finding”
17. Addition of note about Ethiopian study coordinators duty to secure all appropriate IRB approvals
18. Change in Ethiopian study coordinator from Sintayehu Gebresillassie to Mulat Tarekegn

26 February 2016 – Version 2.5 includes the following updates since Version 2.4 (9 October 2015):

1) Addition of the SWIFT-STH supplement
2) Addition of other diseases of interest (helminthic, intestinal bacterial pathogens, and intestinal protozoal pathogens) to the serological testing component of the study
3) Addition of stool PCR to protocol – Kristen – 03/01/2016
4) Update blood protocol to reflect training – Kristen 03/02/2016
5) Update stool protocol to include stool PCR (The basis of these updates came from a PLoS paper by Steven A. Williams.)

9-10 March 2016 – Version 2.6.1 includes the following updates since Version 2.5 (26 February 2016):

1) Includes additional updates on the stool PCR protocol.
2) Updated TAITU age groups from 0-5, 6-9 and 10+ to 0-5 (longitudinal) and 8-12

3) Changed Ethiopian study coordinator from Mulat Tarekegn to Solomon Aragie

2 December 2016 – Version 2.6.2 includes the following updates since Version 2.6.1 (9-10 March 2016):

1) Includes addition of rectal swabs for 100 children as an appendix item

25 September 2017 – Version 2.6.3 includes the following updates since Version 2.6.2 (2 December 2016):

1) Updated the cluster sample size numbers.
    - Changed WUHA sample size from 44 clusters with 22 WASH and 22 Delayed WASH clusters to 40 clusters with 20 WASH and 20 Delayed WASH. This decrease in sample size was due to the drought in the study area that was taking place during the identification of eligible clusters. There were fewer clusters available than where initially anticipated due to a decreased water table from the drought.
    - Changed TAITU sample size from 24 with 12 Targeted and 12 MDA clusters to 32 with 16 Targeted and 16 MDA clusters. This increase in sample size was due to the fact that the population size in the TAITU clusters was smaller than anticipated.
2) Changed hygiene cluster coordinators to hygiene cluster coordinator, since only one hygiene cluster coordinator is being employed at this time
3) Added explanation of the Health Promotion Workers and their role in the study throughout the MOP
4) Noted that individuals in the WUHA portion of the study who are 10 or older will only be sampled at baseline and 36 months.
5) Noted that the NP swabs are only being taken at baseline and the endline for each study respectively

19 December 2019 – Version 2.6.5 includes the following updates since Version 2.6.3 (25 September 2017):

- Re-ordered some of the sections to make the MOP more user friendly
- Noted the name change of the Hygiene Officer to Cluster Coordinator
- Updated procedures for data collection and census
- Noted the procedures for water point selection and types of water points added
- Updated WUHA intervention section to align with intervention as it was implemented
- Noted changes to sampling populations in exam section