

Azithromycin Reduction to Reach Elimination of Trachoma (ARRET)

Manual of Procedures

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1. LIST OF ACRONYMS

BUA	Biological Use Authorization
CDC	Center for Disease Control
CO	Corneal Opacity
DBS	Dried Blood Spot
DSMC	Data and Safety Monitoring Committee
DCC	Data Coordinating Center
DNA	Deoxyribonucleic Acid
GPS	Global Positioning System
IRB	Institutional Review Board
MOH	Ministry of Health
MOP	Manual of Procedures
NEI	National Eye Institute
NIH	National Institutes of Health
NRERC	National Research Ethics Review Committee
PCR	Polymerase Chain Reaction
PNSO	Programme National de Santé Oculaire
SAP	Statistical Analysis Plan
TF	Trachomatous Inflammation – Follicular
TI	Trachomatous Inflammation – Intense
TS	Trachomatous Scarring
TT	Trachomatous Trichiasis
TCC	Trial Coordinating Center
USAID	United States Agency for International Development
UCSF	University of California San Francisco
WHO	World Health Organization

2. INTRODUCTION

2.1 EXECUTIVE SUMMARY

We propose a randomized controlled trial of discontinuation versus continuation of annual mass azithromycin distribution in hypoendemic communities of Maradi, Niger. We will randomize communities with up to 20 % TF prevalence following at least 5 years of mass azithromycin distribution to discontinuation or continuation of 3 additional years of annual mass azithromycin distribution.

2.2 STUDY OUTCOMES

2.2.1 PRIMARY OUTCOME

The primary outcome will be ocular chlamydia as assessed by PCR, measured in a population-based sample of 0-9-year-old children at 36 months.

2.2.2 SECONDARY OUTCOMES

Secondary outcomes will include infectious load of chlamydia among 0-9 year-old children infected with ocular chlamydia, conjunctival inflammation as assessed from conjunctival photography, and seropositivity to chlamydia based on antibody response to Pgp3 and CT694 antigens. Infectious load is of interest as children with higher bacterial loads are thought to be more likely to transmit infection. By measuring infectious load, we will be able to estimate community bacterial load in addition to any effect on ocular chlamydia prevalence. Even if prevalence is the same in treated and untreated communities, it is possible that treatment will lower infectious load in individuals with ocular chlamydia.

2.3 METHODS

2.3.1 RESEARCH DESIGN

We propose a community randomized controlled trial to evaluate if mass azithromycin distribution can be discontinued in low prevalence districts. We propose to randomize *grappes* (communities with 200 to 2,000 residents) in Niger to continuation of annual mass azithromycin distribution to the entire community or discontinuation of azithromycin treatment.

2.3.2 STUDY AREA

This study will be conducted in two districts in Maradi Region, Niger. The districts of Guidan Roumdji and Mayahi have approximately 1 million inhabitants. Per the most recent trachoma impact surveys, conducted in July 2018, these districts have TF prevalence up to 20%. Annual mass azithromycin distribution started in 2008 in Guidan Roumdji and Mayahi.

2.3.3 SAMPLE SIZE

40 communities per arm (80 total) will be included in the study. Please see Statistical Analysis Plan (SAP) for same size calculation details.

2.3.4 STUDY PERIOD

The study will take place over a three-year period.

3. ORGANIZATION AND POLICIES

3.1 STUDY ORGANIZATION

3.1.1 STUDY PARTNERS

The Proctor Foundation and PNSO are natural partners to conduct the proposed research. They have worked together on a Gates Foundation-funded cluster-randomized trial of mass azithromycin distribution in Niger. The research partners complement each other, with the Proctor Foundation providing expertise in study design and analysis, and PNSO implementing the fieldwork in a thorough yet efficient manner.

3.1.2 EXECUTIVE COMMITTEE

The Executive Committee will consist of Drs. Catherine Oldenburg and Thomas Lietman from the Proctor Foundation and Prof Abdou Amza from PNSO. This committee will act as the administrative and executive arm of the clinical trial and will

meet in person twice a year to provide overall oversight for the study and make decisions on day-to-day operation issues, including:

- Supervise study progress and data collection progress
- 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. Thomas Lietman will lead the center, which will also include Dr. Catherine Oldenburg (co-PI), Dr. Travis Porco (biostatistician), Dr. Jeremy Keenan (co-investigator), Ms. Fanice Nyatigo (statistical analyst), and Ms. Ariana Austin (Proctor program manager). 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 operations and procedures, obtaining ethical approvals from all involved parties (UCSF, Niger MOH), 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 once per year before each monitoring visit to conduct training sessions with outcome assessors and monitor the quality of data collection. The principal investigators and Proctor program manager will be present at all site visits. The Proctor program manager, with assistance from the PNSO study coordinator, will organize the training sessions. The TCC will meet officially as a group at least four 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 PNSO staff in Niger and expertise with cluster-randomized trials in resource-poor settings.

3.1.4 NIGER COORDINATING CENTER

The Niger Coordinating Center will be located at the PNSO headquarters in Niamey, Niger. The center will be led by the study site principal investigator, Prof. Abdou Amza, and also include Nassirou Beido, the PNSO study coordinator. Prof. Amza will oversee the study activities that take place in Niger and will manage the day-to-day activities of the PNSO study coordinator. He will also assist with obtaining ethical approval from appropriate federal, regional, and zonal agencies. The PNSO study coordinator will work with the local health officials to find health extension workers for the census activities and nurses to work as data collectors. He will supervise all data collection activities and will be responsible for proper storage and transport of specimens.

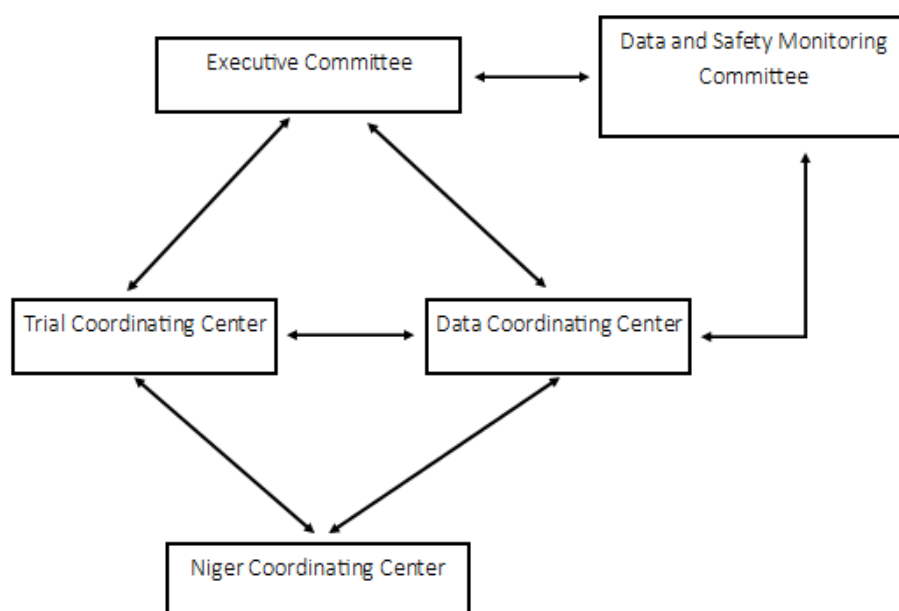
3.1.5 DATA COORDINATING CENTER (DCC)

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 the cluster-randomized trials of trachoma in Ethiopia (TANA and continuation study, NEI U10 EY016214) and Niger (MORDOR; Gates OPP 1032340), and individually randomized trials of corneal ulcer treatment and prevention in India (SCUT, NEI U10 EY015114; MUTT, NEI U10 EY018573) and Nepal (VIEW, NEI U10 EY022880). Dr. Porco, along with Ms. Fanice Nyatigo (statistical analyst) will be responsible for data management, data quality control, event adjudication, and training and certification of enumeration 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

Board, 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 meet monthly. The DCC will be in close contact with the Proctor study coordinator, who will help address any issues with her counterpart in Niger.

3.1.6 DATA SAFETY AND MONITORING COMMITTEE (DSMC)

The DSMC will be formed according to NIH guidelines and is comprised of independent experts in bioethics, biostatistics, epidemiology, ophthalmology, and global health. The DSMC will meet at least once per year. Ad hoc meetings may be convened as necessary. The DSMC will approve the trial protocol at the first meeting, or recommend modifications to the protocol as necessary. At annual meetings, the DSMC will review data on efficacy outcomes. They will also monitor for unanticipated events.



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 more than 60-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 the 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 other parts of the world. From this initial inception, the Foundation has expanded 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. Catherine Oldenburg and Dr. Tom Lietman, the Principal Investigators of this study at the Proctor Foundation, will be assisted by several co-investigators, one study coordinator, a microbiologist, a data management specialist, laboratory PCR processing staff, and a biostatistician.

3.2.2. PROGRAMME NATIONAL DE SANTE OCULAIRE (PNSO)

The Programme National de Sante Oculaire is an institution housed in the Ministry of Health in Niamey, Niger. PNSO is responsible for development and evaluation of the National Eye Health Program of Niger, with the goal of reducing visual impairment and the prevalence of blindness in Niger. PNSO is also involved in research related to preventable blindness in Niger. The coordination team of PNSO consists of 3 ophthalmologists, 1 manager, 1 coordinator, 1 epidemiologist, and 2 drivers on the full-time staff. PNSO has administrative and financial offices, a data management unit, and has close ties to district hospitals in Maradi and other regions throughout Niger. The capacity and experience of the personnel at PNSO and their close connections with communities involved in the project will ensure the success of proposed work.

3.3 DUTIES AND RESPONSIBILITIES OF STAFF

3.3.1 PRINCIPAL INVESTIGATORS (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, develop grant budget
- Ensure that staff follow through on protocol and properly execute all areas of research
- Ensure that all ethical approval is maintained
- 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 trachoma examiners

3.3.2 STUDY COORDINATOR (PROCTOR FOUNDATION)

- Ensure the execution of the study per protocol
- Coordinate with the collaborating center, PNSO, particularly with the PNSO Study Coordinator in execution of the study
- Manage correspondence between all collaborating organizations and parties
- Maintain all ethical clearances for the study, including IRB renewals, NRERC, and BUA and DSMC-related approvals
- In collaboration with PNSO Study Coordinator, prepare all forms and documents necessary for fieldwork (randomized registration forms, exam sheets, fieldwork documents directing team, etc)
- Train census workers in the use of electronic data capture system (with help of PNSO Study Coordinator)
- Train both Proctor and Nigerien health worker teams for each collection visit; direct team (with help of PNSO Study Coordinator) while in Niger
- Arrange logistics and itineraries for traveling team members in Niger
- Purchase, maintain, and organize transport of all necessary study supplies to/within Niger
- Maintain communication and partnership with Principal Investigators regarding all study activities and plans

3.3.3 STUDY COORDINATOR (PNSO)

- Prior to the start of the study, secure support from the Ministry of Health
- At baseline, obtain permission from the district health leaders; meet with and obtain written consent from all community leaders

- Supervise and train collection team (with emphasis on any new members) throughout each collection, especially after departure of Proctor team
- Train and supervise all census taking teams, including training in the electronic data capture system
- Charge all tablet computers and battery packs each night of the census
- Train and supervise all Zithromax distribution teams
- Maintain all fieldwork documents and records
- Complete all collection and intervention reports
- Work with Data Coordinator to maintain treatment and census database
- Oversee transport of study samples
- Assist Proctor Coordinators in coordinating logistics in Niger
- Train and supervise enumeration staff at PNSO in Niamey
- Analyze and provide data when requested by co-investigators or staff from Proctor
- Appropriately back up all data

3.3.4 CO-INVESTIGATORS (UNITED STATES AND NIGER)

- Assume responsibility for the study in the absence of the Principal Investigators
- Supervise ophthalmic assistants, ophthalmic nurses, local health agents, and other Proctor field team members in the field to ensure conformity to study procedures
- Communicate with Study Coordinator and Principal Investigators to ensure the execution of the study per the protocol

3.3.5 STATISTICAL ANALYST (PROCTOR FOUNDATION)

- Create and maintain the database for all collection and results-related data for the study:
 - Monitor correct receipt of data after each collection visit
 - Develop consistency checks in the data management – verify any inconsistencies or question 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

3.3.6 BIostatistician (PROCTOR FOUNDATION)

- With the Principal Investigators, create the Statistical Analysis Plan (SAP)
- Receive all study data and review for quality control purposes
- Ensure appropriate masking
- Prepare data analysis plan for DSMC meetings; oversee analysis and prepare all presented data for DSMC meetings, DSMC reports, and all study publications

3.3.7 TREATMENT AND CENSUS TEAM

- PNSO will hire and train “health extension workers” to perform the census

- During census phase, travel to the 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 member:
 - Name
 - Gender
 - Age
 - GPS coordinates of each household
 - GPS coordinates for each safe water source in the study area
 - GPS coordinates for each school in the study area
- During the treatment phase:
 - Complete training by PNSO Study Coordinator and officials from District Health Department regarding proper treatment protocols and procedures
 - Travel to enrolled clusters and administer study medication to the study subject per protocol
 - Directly observe consumption of study drug
 - Record antibiotic coverage against previous census
 - 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 participants, and report this information immediately to PNSO investigators

3.3.8 COLLECTION TEAM

- At each collection 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 PNSO 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 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 participants, and immediately report to PNSO investigators

3.4 POLICY MATTERS

Any changes to the protocol made during the course of the study will be incorporated into the revised protocol and the Manual of Operations and Procedures (MOP) and recorded in a change log. Any new forms will be incorporated as an 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 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) as well as the DSMC.

4. PARTICIPANT FLOW

4.1 ELIGIBILITY REQUIREMENTS

4.1.1 INTERVENTION ELIGIBILITY

All individuals in all clusters will be eligible for randomization to continuation or discontinuation of mass distribution of azithromycin.

4.1.2 MONITORING ELIGIBILITY

Monitoring will be based on population-based samples. The following groups will be monitored at each study visit: 1) a random sample of 50 children aged 0-9 years will receive conjunctival photography and swabbing; 2) a random sample of 40 children aged 1-9 years will receive dried blood spot collection. Eligibility for these groups will be based on the previous census.

4.2 RANDOMIZATION

4.2.1 CLUSTERS

The randomization unit for this trial will be the grappe.

4.2.2 CONTAMINATION

Cluster-randomized trials are subject to contamination between clusters, which could weaken observed effects of the intervention if intervention activities occur in non-intervention clusters. Contamination will be minimized in this study because study activities (e.g., census and treatment) will not be available in areas not randomized to the intervention. We also reduce the chances of contamination by choosing relatively large geographic area, the grappe, as the randomization unit, and by choosing a sentinel development team for monitoring in each grappe from within this unit. This increases the distance between clusters and creates a buffer zone that reduces admixture between intervention and control groups.

4.2.3 TREATMENT ARM RANDOMIZATION

Study communities will be randomized into one of the 2 study arms after the baseline monitoring visit. Randomization will occur after the baseline monitoring visit to reduce the potential for 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 MASKING

Given the nature of the intervention, participants and investigators will not be masked as to study arm. Census workers who conduct the census will be masked to treatment arm of the study community, as will outcome assessors conducting clinical examinations and collecting swabs. Laboratory personnel processing conjunctival swabs for ocular chlamydia and dried blood spots for serology will be masked to treatment group and to control versus study samples. Photographs will be graded by a masked grader. All samples and photographs will be processed in a random order. Masking will not only minimize bias introduced through knowledge of treatment assignment but will ensure that the census and outcome assessment is identical in all study communities.

5. CENSUS

We will perform a house-to-house census of the selected study communities at baseline prior to randomization. The census will be updated at 36 months. PNSO will organize and train existing health extension workers to perform the census. Health extension workers in Niger are experienced in census-taking, and PNSO has experience in conducting large censuses in Niger, as they conduct a census every year. We will use an electronic data capture system for the census, as is currently being used in the Gates Foundation funded MORDOR Burkina Faso study. This system utilizes tablet computers to record all census data. Census workers will record the name, gender, and age of each household member. Each tablet has GPS capability, and coordinates will be recorded for each household in the study area with the tablet.

5.1 EQUIPMENT

Each census worker will be given the following equipment:

1. Tablet computer: LG Stylo 5 (1)
2. Battery pack: New Trent iCarrier 12000 mAh Dual Ports 2A/1A charger (1)
3. Charging cord (from charger to electrical socket) (1)
4. Connecting cord (from computer to charger) (1)
5. Speck protective case (1)
6. Sealable canvas bag to hold all equipment (1)

5.2 MOBILE APPLICATION

A custom-made software application, which runs on the Android platform, will be used for data collection in the study. The software application will be created through CommCare. Census workers will enter all data directly into the application.

5.3 TRAINING

The study coordinators from the Proctor Foundation and PNSO will run a 2-day training workshop for all census takers to train them in the following topics:

1. General use of tablet computers
2. Entering data into the mobile application
3. Acquiring GPS coordinates with the device
4. Re-charging the device

5.4 CHARGING DEVICES

The PNSO coordinator will be responsible for charging all devices each night. This will be done at the coordinator's hotel, which must be equipped with a backup generator. The study coordinator will be charging 20 devices and 20 chargers per night, so will bring sufficient power strips for this task.

6. STUDY INTERVENTIONS

The cluster-randomized trial consists of two arms: 1) annual mass azithromycin distribution to all residents per WHO guidelines (*Continue Treatment*); 2) discontinuation of mass azithromycin distribution (*Stop Treatment*).

6.1 INTERVENTION

6.1.1 MASS AZITHROMYCIN DISTRIBUTION

In all study communities randomized to the annual treatment arm, all individuals aged 1 month and older will receive a single mass distribution of azithromycin several weeks after the baseline monitoring visit, and then annually for 3 years. Oral azithromycin, 20 mg/kg for children and 1 g for adults, will be offered to all households identified on the preceding census in the communities randomized to continuing treatment. Study drug will be distributed by health extension workers and organized by PNSO. Individuals with a known macrolide allergy will be offered a two-week course of daily ophthalmic tetracycline ointment (two tubes).

6.2 TRAINING

Census trainers, antibiotic distributors, hygiene officers, and data collectors will be trained prior to each round.

6.2.1 FIELD STAFF: CENSUS

Field staff will be trained at a two-day workshop immediately before the monitoring visit starts. Field workers will be trained in the use of the electronic data system including the mechanics of the data collection software, the proper handling of the devices, and the charging of the batteries. Before being certified to collect data, census workers must pass a practical examination conducted by the Proctor study coordinator. Field staff will be trained in making the oral antibiotic suspension and completing the study documentation.

6.2.2 FIELD STAFF: SAMPLE COLLECTION

Field staff will be trained at a two-day workshop immediately before the monitoring visit starts. Field staff will receive extensive training in conjunctival swabbing, and we will especially emphasize the danger of contamination of ocular swabs. Several field staff members will be recruited to take conjunctival photographs with a smartphone and 3D printed cellscope. Field staff will only be certified to photograph, or swab if they pass a practical examination conducted by the Proctor study coordinator.

7. MONITORING

We will perform monitoring visits in all study clusters. Monitoring for outcome assessment will occur in the sentinel surveillance development teams for all grappes and will be identical across all study arms.

7.1 OUTCOME MONITORING

Outcome monitoring includes monitoring for all outcomes, including ocular chlamydia prevalence, and clinically active trachoma. Outcome monitoring will occur via an identical protocol for all development teams in all grappes, regardless of randomized study arm. The population-based sample in the sentinel development team will consist of an age-stratified cross-sectional sample at each monitoring visit. Eligibility will be based on the most recent census. We will sample a 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.

8. EXAMINATION PROCEDURES

8.1 MOBILIZERS

The PNSO study coordinator will hire a community member from each monitored community to mobilize the selected children and ensure a high turnout for the monitoring visits. The person will also select the location for the monitoring visit.

8.2 OUTCOME ASSESSORS

In collaboration with district health officials, the PNSO study coordinator will hire clinical nurses and public health nurses from a neighboring district to perform outcome assessments. The nurses will receive a two-day training session at the beginning of the monitoring visit to review all study procedures. Nurses will be masked to treatment allocation.

8.3 REGISTERING PARTICIPANTS FOR EXAMINATION

Prior to clinical examination the Registration Assistant will identify the participant, verify his/her age, and assign the next random identification number from the randomization list. Random numbers will be assigned for laboratory and database anonymity. This random identification number will be entered into a tablet computer in order to link the laboratory specimens with the unique identification number used for the study.

8.4 GLOVING OF THE EXAMINERS HANDS

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

8.5 EXAMINATION POSITIONS

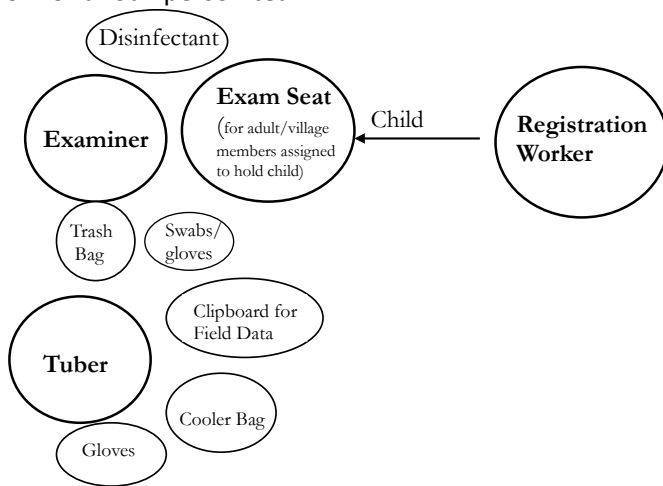
The examining position to be used in the field for young children will be the classic pediatric ophthalmic examination technique. With the aid of a helper seated directly opposite the examiner, the child will be positioned with his/her head between the examiner's knees, with the child's face looking upwards towards 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.

For examining 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.

8.6 FIELD SPECIMEN COLLECTION QUALITY CONTROL MEASURES

The stations for examination and swabbing of participants are displayed below. This setup is designed for the most efficient patient flow and to minimize errors or confusion between team members. This diagram shows the station configuration for a four-person team.



8.7 CONJUNCTIVAL SWAB COLLECTION

A random sample of 50 children <10 years old will undergo conjunctival swabbing at 0 and 36 months. Swabs will be processed for *C. trachomatis*. Samples will be processed within in pools of 5. Any positive pools will be unpooled and processed individually. Swabs will be labeled with a unique barcode in the field, which will link to a unique identification number. The number will be linked with the individual's census entry during swab collections in the field.

8.7.1 PROTOCOL FOR COLLECTION

After clinical photography and grading, we will collect 2 conjunctival swabs per child. One conjunctival swab will be stored in Zymo's DNA/RNA Shield media, and one will be stored dry. The examiner will swab the upper right conjunctiva while it is still everted as follows:

1. Pull apart the swab sachet in a sterile manner to reveal only the tip of the swab shaft with the swab head still remaining sterile deep in the sachet.
2. Pull out the swab and hold it no closer than 1 inch from the tip of the swab in order to avoid contamination.
3. Run the swab firmly on the upper tarsal conjunctiva in one direction.
4. Rotate the swab 120° along its axis and rub firmly again. Rotate and repeat a third time.

Materials Needed.

Swabs: Specimens will be collected using sterile, individually-wrapped Dacron polyester-tipped swabs with a plastic shaft (manufactured by Fisherbrand®).

Sample Tubes: All field samples for DNA testing will be collected into sterile DNA-free 2.0ml microcentrifuge tubes, manufactured by Sarstedt®.

Cooler Bags with Frozen Ice Packs: Same as samples for resistance testing.

-20°C Freezer

Protocol for Tubing and Handling of Conjunctival Samples. The swab shaft should only be inserted until the Dacron swab head is fully in the tube. The tuber should lower the cap onto the swab shaft held by the examiner, and the examiner should quickly break the swab shaft using a swift downward snapping wrist movement (this will be demonstrated in the field the first day).

All samples will be in sample boxes, labeled with the village name for easy future identification.

- Study name and visit number: ARRET 0
- Where were samples collected: Village name
- How many samples were collected: Number of swabs and circle the number
- When were samples collected: Date (19SEP2019)
- What type of samples were collected: Dry conj or Zymo conj

Negative Field Controls. Four negative field control swabs will be taken in each community to assess for contamination. Two control swabs are taken before specimen collection begins in a community (one dry, one in Zymo media); and again upon completion of specimen collection (one dry, one in Zymo media).

1. For each negative field control, the examiner will open a new swab as described above.
2. Wave the swab in the air, without making contact with anyone/anything.
3. At the beginning of the day, tube one conjunctival swab in Zymo media, and tube one in an empty, dry tube, following the protocol above.
4. At the end of the day, tube one conjunctival swab in Zymo media, and tube one in an empty, dry tube, following the protocol above.

8.8 FIELD GRADING AND CLINICAL PHOTOGRAPHY

The same random sample of 50 children <10 years old who will undergo conjunctival swabbing will also undergo conjunctival photography and trachoma field grading. Four specific team members will be designated as photographers for the entire trial to ensure high quality photographs by an experienced photographer.

8.8.1 TRACHOMA FIELD GRADING OF THE CONJUNCTIVA

Trachoma field grading will be performed in addition to the conjunctival photography. Field grading will be done before photography or swabbing.

8.8.2 TRACHOMA FIELD GRADING PROTOCOL

First, the examiner will wear the loup and will flip the conjunctiva.

The examiner will flip the child's **right** eyelid:

1. Wearing gloves, the examiner will use his or her fingers to grasp the central part of the child's right upper eyelid.
2. The eyelid is then turned over using the finger of another hand (or the tip of a sterile swab) as a fulcrum.
3. The inverted eyelid is held in place by the examiner's non-dominant hand

The examiner will observe the conjunctiva and will tell the photographer the trachoma grading he found following the WHO simplified trachoma grading scale below

TF: trachomatous follicular	Presence of five or more follicles in the upper tarsal conjunctiva
TI: Trachomatous Inflammation	Pronounced inflammatory thickening of the upper tarsal conjunctiva
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

This grading will be recorded in the mobile application.

8.8.3 CLINICAL PHOTOGRAPHY OF THE CONJUNCTIVA

The photographer will take photographs of the conjunctiva with a handheld smartphone with a 3D-printed cellscope. Conjunctival photography causes no damage to the eye, is well tolerated by children, and is a standard clinical procedure at UCSF. We will use an identical protocol to that being used in the MORDOR study. Clinical photography will be performed before conjunctival swabbing and after field grading. Photographs will be identified by first taking a photograph of the 6-digit random identification sticker that was assigned to the child during the registration phase of the study visit. By convention, all photographs taken after this random number (up to the next photographed random number) belong to the preceding random number. If a random number is mistakenly not photographed before taking a photograph of the conjunctiva, it may be photographed afterwards; in this case, a note should be included that says “preceding photos”. Photographs are taken in the mobile application.

8.8.4 PHOTOGRAPHY PROTOCOL

Equipment needed. Smartphone, 3D-printed cell scope and battery, latex or other sterile gloves

Photograph Procedures.

1. Place the participant into position that will allow maximum stability; standing, sitting, or “head-clamp” position. Employing a village volunteer to help is very useful.
2. The photographer scans the child’s bracelet using the smartphone.
3. The examiner everts the child’s right upper eyelid.
 - a. Wearing latex (or other sterile) gloves, the examiner will use his/her fingertips to grasp the central portion of the participant's right upper lid eyelashes.
 - b. The right 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.
 - c. 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 tarsal conjunctiva. If the right eyelid is difficult to flip because of eye disease or injury, the examiner is allowed to flip the left eyelid instead.
4. The examiner holds the child’s flipped eyelid for the photographer.

5. Take a minimum of two photos. If there is any doubt about the quality of the photo while the participant is in position it is better to continue to take more photographs before the participant is allowed to leave. It is easy to delete photos if they are not needed.
6. Check photos before allowing the child to leave. If they are not acceptable, repeat the procedure. Only stop if the participant or guardian requests that we stop, or if it is deemed impossible, even with further attempts. Note that we have obtained >95% acceptance in previous studies.
7. If the child cannot be photographed for some reason, it does not affect the eligibility of the child.



Smartphone



Cellscope

**The conjunctiva should take up the majority of the space
in the center of the photograph.**

- Conjunctiva is completely everted
- Conjunctiva is centered
- In Focus: If there are blood vessels, they should be focused.
- Entire conjunctiva: Make sure the examiner's glove is not covering part of the everted conjunctiva
- Appropriate brightness: Not too bright and not too dark; no sun in the background

Taking Photographs of the Conjunctiva.

When the examiner has the child's upper eyelid everted:

- Turn on the LED light on the Cellscope
- Activate the camera
- To take a photo of the conjunctiva:
- If necessary, tap the center button to focus
- Remember to turn off the LED light after taking photographs to save battery power.

After photographs are taken, disinfect the part of the cellscope that touches the child's face with an alcohol wipe.

Photo requirement: Take at least two good conjunctival photographs.

8.8.5 GRADING OF CLINICAL CONJUNCTIVAL PHOTOS

Three experienced ophthalmologists will grade the photographs, masked to treatment allocation. We will present graders an ordered set of photographs for each monitored individual, with the study visit identified for each photograph (e.g., baseline or 36 months). Graders will grade each photograph in the set for follicles and inflammation according to the grading scale described by the WHO Simplified Grading System.

8.8.6 PHOTOGRAPHIC READING CENTER

The Reading Center for grading of conjunctival photographs will be based at the Proctor Foundation. 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 single 24" computer monitor (1920 x 1200 pixels, re-calibrated monthly to a color temperature of 6500 Kelvin and 2.2 gamma) 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 PNSO study coordinator will be responsible for renaming each of the photographs taken during the study. The naming convention to be used is that the file will be a concatenation of the 6-digit random number, then the study visit (0, 36), and then the photograph number from that visit. For example, if a child with random number 111999 had 3 photographs taken at the 36-month visit, these 3 photographs would be relabeled as 111999-36-1, 111999-36-2, and 111999-36-3.

Photograph Selection and Arrangement. The best quality conjunctival photograph will be selected from each participant and displayed in a PowerPoint slide (left=month 0; right=month 36). The random number will be added to each PowerPoint slide for identification purposes. The best photograph will be pre-selected but the photographic graders will have access to all other photographs taken of the study participant in a folder on the computer; they simply navigate to the correct file name using the naming conventions listed above.

8.9 DRIED BLOOD SPOTS COLLECTION

A random sample of 40 children 1-9 years old will undergo dried blood collection at 0 and 36 months. The dried blood spots will be processed for *C. trachomatis*. Samples will be processed within in pools of 5. Any positive pools will be unpooled and processed individually. Swabs will be labeled with a unique barcode in the field, which will link to a unique identification number. The number will be linked with the individual's census entry during swab collections in the field.

8.9.1 PROTOCOL FOR COLLECTION

Materials Needed.

FTA Elute cards
Small zip plastic bags
Desiccant packs

Masking tape

Large Ziploc bags (handful)

TropBio circular cards

Materials for drying apparatus: 12 sharpened pencils, Styrofoam, empty cardboard box

Procedures.

Fingerstick. Inform the mother that her child's finger will be pricked to obtain blood. 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.

A finger stick of capillary blood will be collected for dried blood spots to be stored for later testing. Blood will be collected by a gloved health worker using aseptic technique. Gloves will be changed between each participant. The fingerprick or heelstick site will be disinfected using a 70% isopropyl alcohol swab.

Fingerstick procedure:

1. Prepare the disposable lancet. Use a NEW disposable lancet for each child. Do not re-use lancets!
2. The recorder will scan the child's QR code, and place a random number sticker on the TropBio filter paper and the (right edge of the) slide.
3. Position the child for the finger stick. Make sure that the child's right hand is warm and relaxed. Hold the child's thumb, middle, or ring finger on his/her right hand (from the top of the knuckle to the tip of the finger) between your left thumb and finger and disinfect in small outward circles with an individually packaged alcohol wipe.
4. After the alcohol dries, use the thumb to lightly press the child's thumb or finger from the top of the knuckle towards the fingertip to stimulate blood flow towards the sampling point (puncture site). For the best blood flow and least pain, prick the side of the thumb/fingertip, not the center. While applying light pressure towards the thumb/fingertip, hold the lancing device in your hand and prick the thumb/finger. If the finger prick is performed properly, a single prick should be sufficient to collect the required amount of blood.
5. Allow the blood to ooze out. Wipe away the first 2 or 3 drops of blood with gauze. If necessary, re-apply light to moderate pressure towards the thumb/fingertip (approximately 1 cm behind the site of the finger prick) until another drop of blood appears.

Note: Do not squeeze forcefully. Avoid "milking" as it may dilute the blood with tissue plasma.

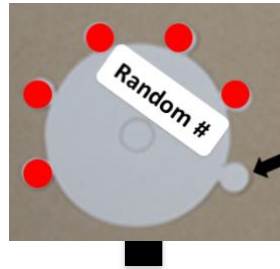
Collection.

Collecting the FTA Elute filter paper sample:

1. Label the filter paper with a random number sticker.
2. Place 2-4 large drops of blood directly from the thumb or finger onto the large circle on the filter paper (if it is difficult to obtain 4 drops of blood, it is sufficient to collect 2 drops of blood).
3. Leave the filter paper to air dry for a few minutes, then place the sample into a small plastic bag along with a desiccant packet.
4. Leave the bag open for a few minutes more, and when the blood is **completely** dry, roll down the top of the bag and close with a piece of masking tape.
5. Store the filter paper samples (in small plastic bags) in a larger Ziploc bag. Keep all filter paper samples in a safe, dry place at room temperature.
6. Blood spots will be stored at room temperature in a locked cabinet in the study coordinators' office.

Collecting the TropBio filter paper sample:

1. Label the filter paper with a random number sticker.
2. Grip the filter paper on the side without small circles. Place a droplet of blood directly from the thumb or finger onto five of the six circles, leaving the right one blank. Be sure to fill each circle completely.



Leave last circle blank

Area to hold the filter paper.
Do not touch the small circles.

3. The recorder will scan the QR code.
4. 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 box or container to protect from dust.
5. When the filter paper is dry, place each sample into a small zip plastic bag (individually). Place the small bags into a larger Ziploc bag with five desiccant packets.
6. Ensure the large Ziploc bag is sealed tightly, as moisture will damage the samples. Transport these filter paper samples to a freezer.

Set-up drying area for TropBio bloodspots.

Supplies: pencils, Styrofoam, cardboard box, paper

- Place Styrofoam in cardboard box
- Put pencils in box/container – space apart
- *Note:* When placing the blood spot samples on the pencil, space apart by ~2.5cm with pieces of paper in between each sample.



9. MICROBIOLOGY LAB PROCEDURES

9.1 MICROBIOLOGY PROCEDURES

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 6-digit random identification number, masking laboratory personnel and preventing identification of the individuals infected. For outcome assessment, laboratory results will not be available for weeks if not months.

9.2 METHODS

Laboratory testing is the current standard of care of identification of *C. trachomatis* infections in the United States. After collection, all samples will be processed in the Ralph and Sophie Heintz Laboratory at the Proctor Foundation in San Francisco and will be filled with 1 mL of M4RT media, and testing for *C. trachomatis*.

Swabs will be processed with the Abbott RealTime assay for *Chlamydia trachomatis*, using the automated Abbott m2000 System. 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).

9.2.1 PROCESSING OF SAMPLES FOR PCR

Samples are handled as per Abbott RealTime sample processing protocol, with the following modifications:

1. 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.
2. Samples are pooled.

9.2.2 PROCEDURE FOR MASKING SAMPLES

In order to mask the location origin, the control status, and the clinical exam grading of the conjunctival samples collected in the field, the Database Manager will assign an identification number different from the random number assigned to each child. The PCR results will be recorded according to this laboratory identification number, thus masking the lab until all the samples have been processed. The Database Manager will then link the lab ID number to the random sample number to reveal the test results by development team.

9.2.3 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. We will unpool all positive pools in the ages 1 month – 9 years 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 ≥ 10 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.

In order to pool the conjunctival samples in the lab, the microbiology lab staff will assign a new pool ID number for every sample, and samples will be stored at -80°C freezer until PCR testing (if not processed that day).

9.2.4 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 RealTime 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.

9.2.5 QUANTIFICATION

For every individual 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.

9.2.6 LABORATORY RESULTS REPORTING

All lab results will be kept in computer files as well as in hard-copy form by the Database Manager. The principal investigators and the DSMC will be updated regularly on the progress of the lab work throughout the course of the study.

10. TREATMENT

10.1 MASS AZITHROMYCIN DISTRIBUTION

All individuals in communities randomized to azithromycin continuation will receive one annual mass distribution of azithromycin after baseline census and monitoring. During the mass treatment, all individuals aged 1 month and older will be offered a single dose of directly observed oral azithromycin (1g for adults and 20mg/kg for children). Children under 1 month, 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 preceding 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.

10.2 ELIGIBILITY

During the mass treatment, all individuals in communities randomized to azithromycin continuation aged 1 month and up will be offered a single dose of oral azithromycin, 20mg/kg for children using height-based dosing and 1g for adults. Those under 1 month 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 study drug as per each study arm outlined above, except those contraindicated by the Ministry of Health, which include:

- Those self-reported as pregnant
- Those known to be allergic to azithromycin or macrolides such as erythromycin

These three exceptions will be treated with topical tetracycline eye ointment. Women uncertain of their pregnancy status may be offered an immediate-result pregnancy test if deemed appropriate by ethics committees.

10.3 ADHERENCE TO TREATMENT

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

10.4 SIDE EFFECTS

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 chairman of the development team, who will then inform the health care representative. This person will in turn inform PNSO site manager, who will contact the Co-Investigator in Niamey. 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 PNSO, 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.

10.4.1 ADVERSE OUTCOMES

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.

10.4.2 PATIENT DEATH

The infant mortality rate is quite high in this area of Niger. 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, receiving Azithromycin treatment may actually have a positive effect.

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

All death records (if available) will be maintained by the Data Coordinator at PNSO. The incidence of mortality for each study arm will be made available to the DSMC by the biostatistician.

11. STUDY MEDICATION

11.1 DESCRIPTION

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

The 1200 mg bottles of azithromycin donated by Pfizer Inc. are specifically labeled, “donation for treatment of trachoma only”.

11.2. DOSAGE INFORMATION

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 PNSO’s program—note that this is supported by the WHO PBD group.

Individuals who are either under the age of 1 month, 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 Niger 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.

11.3 ALTERNATIVE THERAPIES

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

11.4 MEDICATION PROCUREMENT/DONATION

The International Trachoma Initiative will provide the donation of Zithromax® (azithromycin), which will be shipped directly to Niger and received by a representative of the Nigerien 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 Nigerien Customs Authorities and the Nigerien Ministry of Health.

11.5 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 PNSO treatment distribution team.

11.6 MEDICATION QUALITY CONTROL

Study medication will be stored in the PNSO project office prior to use. The hygiene officers 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.

11.7 CHECKING ANTIBIOTIC COVERAGE

In order to observe the study medication coverage of participating development teams, the ophthalmic nurses and public health nurses who participate in the distribution will mark the dorsal aspect of the right hand of all treated individuals in a randomly chosen number of development teams. The treatment distribution team will then return to the treated development team the following afternoon or the next day, to survey every household to determine the number of marked individuals in each development team. This number is then compared to the total population in each development team from the census to establish study medication coverage level. The return visit to the treated development teams provides an opportunity to identify and treat any individuals who missed the antibiotic distribution initially. Workers would return to households until they had distributed antibiotics to at least 80% of individuals.

12. PROTECTION OF HUMAN SUBJECTS

12.1 INSTITUTIONAL REVIEW BOARD APPROVAL

12.1.1 UCSF INSTITUTIONAL REVIEW BOARD

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

12.1.2 Comité d’Ethique du Niger

The study protocol will be reviewed and granted ethical approval by the Ethics Committee of Niger before any study activities begin.

12.2 INFORMED CONSENT

The chairman of each development team will be asked for permission to include the development team in the study. Additionally, the study will be discussed with all adult family members in the development teams by the participating PNSO staff members who speak French or other local language.

At each collection visit, parents/guardians of study participants who are ages 0 – 9 years old will be informed about the possible risks and benefits examination, swabbing, photography, and treatment 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.

12.3 ADEQUACY OF PROTECTION AGAINST RISKS

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

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.

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.

Dried Blood Spot. Blood testing will include a pin prick to the finger or heel. The major risk of this procedure is infection at the puncture site, though using aseptic technique will minimize this occurrence. Individuals in these communities are familiar with this procedure because all children who present at a health center with fever are offered the pinprick for a malaria thick smear.

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.

12.4 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 and children under 1 month will be excluded from receiving study drug, and will be offered topical tetracycline eye ointment in its place.

12.5 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.

13. DATA SAFETY AND 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.

13.1 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.

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.

13.2 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.

13.3 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 to 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.

13.4 TIMING AND PURPOSE OF DSMC MEETINGS

13.4.1 ORGANIZATIONAL MEETING

The initial meeting of the DSMC will be held in September 2020. The committee will provide an advisory review of scientific and ethical issues relating to study design and discuss 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 will be attended by all DSMC members, lead trial investigators, and the trial biostatistician. The DSMC will review drafts of the trial protocol, the Statistical Analysis Plan, and the DSMC Charter. At subsequent meetings, committee members will receive Open and Closed Data Reports.

13.4.2 FUTURE MEETINGS

The initial meeting of the DSMC will be held in March 2020. The committee will provide an advisory review of scientific and ethical issues relating to study design and discuss 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 will be attended by all DSMC members, lead trial investigators, and the trial biostatistician. The DSMC will review drafts of the trial protocol, the Statistical Analysis Plan, and the DSMC Charter. At subsequent meetings, committee members will receive Open and Closed Data Reports.

13.5 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.

13.5.1 CLOSED SESSION

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 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.

13.5.2 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.

13.5.3 OPEN AND CLOSED REPORTS

For each DSMC meeting, Open and Closed Reports will be provided. Open Reports, will include data on recruitment and baseline characteristics, pooled data on eligibility violations, and completeness of follow-up and compliance. The study statistician (TCP) will prepare these Open Reports.

Closed reports, available only to those attending the Closed Sessions of the meeting, will include analyses of primary and secondary efficacy endpoints, including subgroup and adjusted analyses, AEs and symptom severity, and Open Report analyses that are displayed by intervention group. These Closed Reports will be prepared by the study biostatistician.

The Open and Closed Reports 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 at least three days prior to the date of the meeting.

13.6 MINUTES OF THE DSMC MEETINGS

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

13.7 RECOMMENDATIONS TO THE EXECUTIVE COMMITTEE

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. 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 monitoring visits. Study personnel will collect all written data from the census and monitoring visits using tablet computers. This electronic data collection system is currently being used in ongoing trials in Ethiopia and Niger.

14.1 DATA COLLECTION TOOLS

14.1.1 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 census activities, though each census team will also have a backup battery pack. Tablets and batteries will be charged overnight in a stored in a safe, secure place.

14.1.2 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.

14.2 DATA TRANSFER

We will upload the data from the tablet computers directly to a cloud server. The data will be backed up immediately after being uploaded.

14.3 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.

14.4 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.

14.5 DATA COLLECTION

14.5.1 CENSUS ADMINISTRATION

Basic protocols for census taking will be available in French.

14.5.2 FORM DESCRIPTION AND COMPLETING THE CENSUS

The census form that will be used in this study will be prepared by PNSO and printed in French. The census team will visit each development team and inform the chairman 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

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

14.5.3 EXAMINATION AND SWABBING

Data from examination and swabbing is collected on the Registration Form and the Field Form.

Registration Form. The Registration Form is prepared by PNSO and contains the full spelling of each individual's name, age, gender and household number, taken directly from the census.

Field Form. The Field Form contains columns for placing the random numbered stickers for each sample collected in the field, age, gender, indication of photo taken, and clinical exam results for each participant. District and development team name, date, GPS location, and the names of examination team members are also included at the top of each form.

14.6 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.

14.7 ESTIMATION OF DISEASE PREVALENCE

Computerized randomization is utilized to prepare samples for pooling. PCR pooling will be conducted stratified by study community. All samples will be pooled in pools of 5. We will automatically unpool any positive pools results tabulated by development team.

14.8 SAMPLE ORGANIZATION AND STORAGE

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

Samples will be stored at the Ralph and Sophie Heintz Laboratory at the Proctor Foundation in San Francisco in -80° freezers. A protocol to monitor the temperature of the freezer will be established.

15. STATISTICAL METHODS

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

16. MOP CHANGE LOG

Date	Version	Edit