Mycotic Antimicrobial Localized Injection

Manual of Operations and Procedures

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1. INTRODUCTION AND BACKGROUND

Fungal corneal ulcers, because of their poor outcomes and the lack of evidence to guide treatment, present a therapeutic challenge to clinicians.[1] The incidence of fungal keratitis varies by region, which is thought to be due to differences in climate as well as risk of corneal trauma.[1,2] In the tropics, fungal infection can account for upwards of 50% of corneal ulcers.[1,3,4] In the United States fungal keratitis ranges from 35% of corneal ulcers in South Florida[5] to 4% in temperate climates such as Los Angeles.[6] The Mycotic Ulcer Treatment Trial I (MUTT I), a NEI-funded randomized double masked clinical trial found that natamycin is superior to voriconazole for the treatment of filamentous fungal ulcers and in particular for *fusarium* species.[7] However, natamycin is fungistatic and has limited penetration into the corneal layers.[8]

Voriconazole may still be an important adjunct in the treatment of fungal ulcers. *In vitro* studies suggest that voriconazole should have good efficacy against *Aspergillus* and *Fusarium*.[9] Intrastromal injection of voriconazole may provide steady state drug concentrations at the site of infection and avoid intervals of sub-theraputic drug dosing. Secondly, the identity of the organism may play an important role in guiding treatment. Ulcers caused by *Aspergillus* species did equally well with topical voriconazole and natamycin, whereas those caused by *Fusarium* species had 4 lines worse visual acuity at 3 months. Possibly, *Aspergillus* ulcers will fare better when oral voriconazole is added. Thirdly, there may be presenting characteristics, such as deep stromal involvement, which could predict a benefit from intrastromal voriconazole injection.

Studies of intrastromal injection of voriconazole have yielded mixed results. Several case series have found intrastromal voriconazole to be beneficial.[10-12] However, one randomized controlled trial, comparing intrastromal injection to topical voriconazole found significantly improved 3-month visual acuity in the topical voriconazole group.[13] However, in the intrastromal voriconazole arm there were more central ulcers than in the topical voriconazole arm and scar size between the two groups was comparable implying that intrastromal injection does not lead to worse scarring. In this study we will evaluate the effectiveness of intrastromal voriconazole injection in addition to topical natamycin treatment for the treatment of moderate to severe fungal keratitis.

Research Question: Is intrastromal injection of voriconazole beneficial in addition to topical natamycin in the treatment of filamentous fungal corneal ulcers?

1.1 Specific Aims:

1) **Does the addition of intrastromal injection of voriconazole decrease the rate of repeat 3-day culture positivity compared with medical management?** We hypothesize that there will be decreased 3-day repeat culture positivity in the intrastromal voriconazole injection group.

2) **Does intrastromal injection of voriconazole improve best spectacle corrected visual acuity at 3 months compared with medical management?** We hypothesize that intrastromal injection with voriconazole will improve 3-month visual acuity compared with topical natamycin alone.

3) **Does the addition of intrastromal injection of voriconazole improve 3-month scar size, days to re-epithelialization and rate of perforation/need for Therapeutic penetrating keratoplasty (TPK) compared with medical management?** We hypothesize that there will be improved 3-month scar size and decreased perforation rate in the intrastromal voriconazole group, but no difference in days to re-epithelialization.

1.2 Study Outcomes:

Primary Outcome:

- Microbiological cure on 3-day repeat cultures

Secondary Outcomes:

- BSCVA at 3 months
- Microbiological cure on 7-day repeat cultures
- Scar size/depth, as measured by clinical exam, slit lamp photographs and OCT
- Adverse events including rate of perforation/need for TPK
- Thinning as measured by pachymetry and OCT
- Topography
- Difference in IND-VFQ at 3 months between groups
- Corneal neovascularization, as measured by clinical exam & slit lamp photographs

1.3 Study Design:

Mycotic Antimicrobial Localized Injection (MALIN) is a randomized, masked, two-arm clinical trial. The purpose of the study is to determine differences in microbiological cure for 3-day repeat cultures between different antifungal treatments. There will be 1:1 randomization to one of these two treatment groups: 1) Topical natamycin plus intrastromal voriconazole injection or 2) Topical natamycin alone. The enrollment period will be 3 months.

2. ORGANIZATION

Aravind Eye Hospital, Pondicherry along with the University of California, San Francisco (UCSF) will jointly execute this clinical trial. Aravind Eye Hospital, Pondicherry will mainly be responsible for recruitment and enrollment, intervention implementation, and follow-up visits. UCSF will take the lead on all data analysis, writing of study-related materials, and writing journal publications.

2.1 Collaborating Institutions:

Aravind Eye Hospital, Pondicherry, India

Dr. Shivananda Narayana will be the lead investigator and Drs. Tiruvengada Krishnan and Seema Prabu will be the co-investigator for this study in Pondicherry. Administration for the Pondicherry site will be done through Aravind Eye Hospital, Madurai, under the direction of Dr. N. V. Prajna. All recruitment, treatment/intervention, and follow-up visits will be done at the Aravind Eye Hospital at Pondicherry. All study personnel assisting with the research will be adequately informed about the protocol, the research procedures, and their duties and functions through ongoing communication between the 2 sites.

Aurolab at Aravind Eye Hospital in Madurai will be responsible for preparing and distributing study medications.

The microbiology lab in Pondicherry will perform the cultures and serve as a bank for all specimens collected. Madurai microbiology lab will serve as a reference lab that will assist with any difficult microbiological identification.

Francis I. Proctor Foundation, University of California, San Francisco, USA

The Proctor Foundation is an organized research unit at the University of California, San Francisco. The Foundation has a 56-year history of research in ocular infectious and inflammatory disease 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 other parts of the world.

UCSF will perform the data analysis. UCSF will not consent any participants, perform surgeries, or collect study data. UCSF will have the most current protocol, consent documents and HIPAA authorization for reference. All modifications to either IRB of record will be communicated between sites. UCSF will ensure protection of all study-related data by using de-identified information over a secure server. All non-compliance with the study protocol or applicable requirements will be reported in accordance with local policy. There will be ongoing communication of problems, interim results, and study closure between both sites.

Jennifer Rose-Nussbaumer, MD is the co-PI at UCSF for this study. Dr. Rose-Nussbaumer is a Cornea fellowship trained Ophthalmologist at F. I. Proctor Foundation. In addition to her clinical work in cataract and corneal transplant surgery, she is an NIH funded clinical researcher. She is studying corneal ulcer treatment in India and Nepal and corneal transplant outcomes in Ethiopia. She is the principle investigator on the Corneal Preservation Time Study (CPTS) and Descemets Endothelial Thickness Comparison Trial (DETECT) at UCSF. Her previous vision research in Ophthalmology includes work with the World Health Organization on Trachoma, as well as investigating the ocular manifestations of HIV disease. Dr. Rose-Nussbaumer's role in this study will be to perform data analysis, to collaborate with surgery site and eye bank regarding study design and protocols.

3. PATIENT FLOW

3.1 Study Timeline

The enrollment period will be 3 months. Study participants will be required to have 4 follow-up visits at 3 days, 7 days, 1 month, and 3 months (visit window: 2.5 -3.5 months). All study participants will be admitted to the hospital for 7 days. Additional visits may be needed and will be determined by the physician/investigator.

3.2 Eligibility Requirements

Only those who meet all of the inclusion criteria and none of the exclusion criteria will be enrolled in this study.

Inclusion criteria:

- Moderate to severe corneal ulcer that is smear positive for filamentous fungus
- Pinhole visual acuity worse than 20/70 in the affected eye
- Age 18 years or greater, and less than or equal to 70 years
- Basic understanding of the study as determined by the physician
- Commitment to return for follow up visits

Exclusion criteria:

- Gram stain positive for bacteria or evidence of other concomitant infection (i.e. herpes, acanthameoba)
- Impending or frank perforation at recruitment
- Involvement of sclera at presentation
- Non-infectious or autoimmune keratitis
- History of corneal transplantation or recent intraocular surgery
- No light perception in the affected eye
- Pinhole visual acuity worse than 20/200 in the unaffected eye
- Pregnant women
- Participants who are decisionally and/or cognitively impaired

3.3 Randomization

Each study eye will be randomly assigned to the 1) Intrastromal voriconazole plus topical natamycin or 2) Natamycin alone. Block randomization will be performed using a computer program (Statistical package R; Version 2.12; R Foundation for Statistical Computing, Vienna, Austria) by the coordinating site.

Once an eye is enrolled in the study, the study coordinator will assign the study participant's eye an ID (alpha-numeric code) and organize the procedure in the operating room within 24 hours. Once the study participant has been assigned a study participant ID and randomized to treatment group, they will be included in the intention to treat analysis.

3.4 Study visits

3.4.1 Baseline Visit. During this visit, eligible patients at Pondicherry will be enrolled in the study and will give consent. The baseline patient form will be completed and BSCVA obtained. All study participants will begin topical therapy with hourly 5% natamycin immediately. They will have baseline photography and OCT. They will then be randomized to either intrastromal voriconazole plus natamycin or topical natamycin alone. Those randomized to intrastromal injection will undergo this procedure in the operating theatre within 24 hours. They will have an additional 2 stromal injections: one on the 3rd day and one on the 5th day. All patients will be admitted to the inpatient setting until the 7th day.

3.4.2 Procedure visit. Within 24 hours of the baseline visit study participants randomized to intrastromal voriconazole will undergo injection in the operating theatre. They will have 2 additional rounds of intrastromal voriconazole injections: one on the 3rd day and one on the 5th day of the study.

3.4.3 Day 3 visit. During this visit, study participants will have slit lamp examination and undergo repeat scraping and culture of the corneal ulcer. Adverse events will also be assessed and recorded. Thereafter, all additional treatments including but not limited to topical voriconazole, or oral ketoconazole, will be at the clinician's discretion. The treating physician will remain masked to treatment arm. After the scraping and culture have been performed, participants randomized to receive intrastromal voriconazole injections will receive an additional injection of voriconazole in the operating theatre.

3.4.5 Day 5 visit. On day 5 participants randomized to receive intrastromal voriconazole injections will receive their final injection of voriconazole in the operating theatre.

3.4.6 Week 1 visit. Approximately one week from enrollment patients will undergo repeat scraping and culture. No other study visit information will be gathered during this visit, therefore any further examination, testing, or treatment will be at the discretion of the treating physician. After the Day 7th day, patients may be discharged from the inpatient setting.

3.4.7 One Month visit. During this visit, study participants will have their BSCVA, slit lamp examination, and OCT performed during this visit. Photography will also be performed. Adverse events will be reviewed and recorded.

3.4.8 Final Visit. At the 3- Month visit study participants will have their BSCVA and slit lamp examination recorded, in addition to repeat photography, topography and OCT and be administered the IND-VFQ. Endothelial cell count will also be measured. Adverse events will be reviewed and recorded.

3.5 Study Schedule

	Visit 1 Baseline Exam	Visit 2 Procedure	Visit 3 Day 3	Visit 4 Day 5	Visit 5 Week 1	Visit 6 Month 1	Visit 7 Month 3
Forms							
Consent and Authorization form	X						
Baseline form	X						
Procedure form		X	X	X			
IND-VFQ							X
Follow-up form			X		X	X	X
Final form							X
Procedures							
Intrastromal		X	X	X			
Smear/culture	X		X		X		
Tests							
IOP	X		X			X	X
Topography							X
OCT	X					X	X
Slit lamp photography*	X					X	X
BSCVA/ETDRS/MRx	X					X	X
Endothelial cell count							X
Total visit time	2 hours	2 hours	2 hours	2 hours	1 hour	1 hour	1 hour
* Slit lamp photography also taken upon an adverse event							

3.6 Adverse Events (AEs)

During each study visit, the subject will be questioned about AEs in a non-leading manner. All AEs, whether observed by the Investigator, elicited by the Investigator, or spontaneously reported by the subject, will be documented in the subject's chart and the adverse event form. Slit lamp photography will also be completed after any adverse event takes place.

All AEs should be followed until they are resolved or until a stable clinical endpoint is reached. Each AE is to be classified by the Investigator as SERIOUS (SAE) or NONSERIOUS (NSAE).

If a SAE occurs, the investigator at Aravind Eye Hospital, Pondicherry must fill out the serious adverse event form and email it to Stephen McLeod at UCSF within 24 hours of the occurrence of the SAE. The investigator must provide written follow-up reports until the SAE or clinically significant AE has resolved or until a stable clinical endpoint is reached. Notification of an SAE or clinically significant AE must also be submitted to the Institutional Review Board (IRB)/Ethics Committee (EC) in accordance with its requirements. All AEs must be reported from the time that the subject provides informed consent through the last study visit.

4. PROCEDURES

4.1 Masking

All study participants will be masked to their intervention. The refractionist performing the BSCVA will also be masked. Due to the nature of the intervention, the surgeon and technician performing intrastromal injection will not be masked, however, the physician performing repeat scraping as well as the microbiologist will be masked to treatment arm. UCSF study personnel will not be given any identifying information.

4.2 Ulcer Classification

Factor	Mild	Moderate	Severe
Location	Nonaxial	Central or peripheral	Central or peripheral
Area	2 mm	2–6 mm	≥6 mm
Depth	Superficial one third of stroma	Superficial two thirds of stroma	Extending to inner one third of stroma and/ or endothelial plaque

Any two of three criteria should be fulfilled.

4.3 Medical management protocol

In the first 3 days all patients will receive hourly 5% Natamycin, 0.5% Moxifloxacin for prophylaxis every two hours while awake, and 2% homatropine TID. They will be randomized to intrastromal injection with voriconazole versus sham injection. This study requires 7 visits over 3 months including baseline visit, procedure visit, 3-day follow-up and procedure visit, 5-day procedure visit, 1-week follow-up, 1-month and 3-month follow-up. After the 3-day repeat culture visit additional treatments may be added per clinician preference including but not limited to topical voriconazole and oral Ketoconazole.

4.4 Procedures

4.4.1 Corneal Gram Stain and Culture

After patient history and slit lamp examination at the enrollment visit (Day 0), corneal scraping is performed to determine the eligibility of the patient (positive fungal stain and negative bacterial stain). Corneal scraping is recommended as standard of care for all corneal ulcer patients at the Aravind Eye Hospitals. There is a small risk to patients of inducing a perforation, worsening an epithelial defect, or contaminating an ulcer, and patients are duly informed of these risks prior to the taking of the corneal scrape.

Corneal scraping will be obtained as follows:

- A drop of topical anesthetic (0.5% tetracaine or 4% lidocaine) is administered to the eye to be examined.
- Aseptic technique is used to obtain each corneal scrape. A flame-sterilized Kimura spatula is used, with the aid of slit lamp magnification, to obtain a scrape from the leading edge and base of the corneal ulcer. The Kimura spatula is again flame sterilized between the takings of each sample.
- Two scrapings are smeared directly on to two separate glass microbiology slides for Gram stain and for KOH wet mount (if necessary, Giemsa or Gram Stain can be used to identify fungal elements as well).
- Three further scrapings are taken and directly inoculated on to sheep's blood agar, chocolate agar, potato dextrose agar or Sabouraud's agar for bacterial and fungal culture.

4.4.2 Intrastromal Voriconazole Injection

The intrastromal injection will be performed in the operating theatre under the operating microscope by the cornea surgeon under peribulbar anesthesia and with full aseptic precautions. This will be performed within 24 hours of the baseline visit. Voriconazole 0.5 mg/ml solution will be loaded in a 1 ml tuberculin syringe with a 30-gauge needle. With the bevel down, the needle will be inserted obliquely starting in the adjacent uninvolved stroma to reach the infiltrate at the mid-stromal level (as the intended level for drug deposition) in each case. The drug is then injected and the amount of hydration of the cornea is used as a guide to assess the area saturated with medication. Once the desired amount of hydration is achieved, the plunger is withdrawn slightly to ensure discontinuation of the capillary column and thus prevent back-leakage of the drug. Four to six divided doses are given around the infiltrate/s to surround the entire circumference of the lesion.

4.4.3 Additional Injections

An additional two rounds of intrastromal injections will be performed at 3 days and 5 days post enrollment in the voriconazole injection arm only. This MUST be performed after the 3-day repeat culture visit. The study coordinator and treating physician will be aware of the treatment assignment; however, the follow-up physician and microbiologist will remain masked to treatment arm.

4.4.4 TPK

For patients requiring TPK, the corneal button will be sent to microbiology and pathology. Patients should be photographed prior to surgery.

4.5 Anterior Segment Optical Coherence Tomography Protocol

OCT will be documented at baseline, 1 and 3 months. The images will be captured with the Visante using standardized protocols by study certified ophthalmic technicians. The data will then be shared with UCSF via Dropbox.

4.5.1 OCT Scan Procedures

In order to obtain the highest quality anterior segment optical coherence tomography (OCT), the technician will scan the appropriate study eye according to the Visante instruction manual.

All OCT scans will be performed in a designated diagnostic room with a uniform ambient light level.

An initial scan of the global pachymetry will be obtained. Three scans should then be obtained at the pupillary geometric center on the Quad setting (4 line scans through the cornea). Subsequently, a dense scan centered over the area of greatest thinnest (as determined by the global pachymetry) will be performed using the Quad setting. Quantitative data will be obtained using the caliper function by an examiner masked to the results of clinical examination.

4.5.2 Saving Scan

- Save the raw images on the Visante OCT machine
- Download PDF images and save to a USB drive
    - Images over the area of thinning should include the caliper measurement
- De-identify images
    - Patient names or any other identifying information such as birthday should not appear within any of the image files.
- Rename data
    - The image files will be designated with: 1) the study ID number, 2) the timing of visit '1d', "1m', or '3m' respectively for the 1 day, 1, and 3-month follow-up visits, and 3) image number of the series. An image renamed to C101_3m_1.jpg will be the first image for eye number C101's 3 month follow-up visit. An image renamed to C222_1m_3.jpg will be the third image for eye 222's 12 month follow-up visit.
- Batch upload images to Dropbox. Procedures for uploading images to Dropbox:
    - Nagivate to Dropbox → eye's folder → relevant study visit
    - Upload images to the eye's relevant study visit folder

5. STUDY MEDICATIONS

The following medications will be administered for treatment purposes: Natamycin 5%, Moxifloxacin 0.5%, Homatropine 2%, and Voriconazole 0.5mg/ml injection.

5.1 Treatment schedule for study medications

In the first 3 days all patients will receive hourly 5% Natamycin drops, 0.5% Moxifloxacin drops for prophylaxis every two hours while awake, and 2% homatropine drops TID. The aforementioned drugs will be used for FDA approved indications. Study participants will then be randomized to intrastromal injection with voriconazole plus natamycin versus natamycin. All medications will be protected and only appropriate personnel will administer the drugs.

Table 2: Dosing schedule for Medications

Medication	Day 1-3	Day 1 Procedure	Day 3 Procedure	After Day 3 Scraping	Day 5 Procedure
Natamycin 5%	Every hour			Physician determined	
Moxifloxacin 0.5%	6 times/day			Physician determined	
Homatropine 2%	3 times/day			Physician determined	
Voriconazole 0.5 mg/ml		50mg/0.1ml administered via intrastromal injection	50mg/0.1ml administered via intrastromal injection	Physician determined	50mg/0.1ml administered via intrastromal injection

5.2 Voriconazole Dosage and Administration

Voriconazole is an FDA approved triazole antifungal agent. The primary mode of action of voriconazole is the inhibition of fungal ergosterol biosynthesis by inhibiting the cytochrome P450 mediated 14 alpha-lanosterol demethylation. Voriconazole has demonstrated *in vitro* activity against *Apsergillus* and *Fusarium* species and is an approved treatment for serious fungal infections. Intrastromal Voriconazole injection is practice for the treatment of moderate to severe filamentous fungal corneal ulcers at Aravind Eye Hospital, Pondicherry.

Voriconazole is available as 1 mg of white lyophilised powder in a glass vial (VOZOLE PF; Aurolab, India). The powder is reconstituted with 2 mL of lactated Ringer's solution to a concentration of 0.5 mg/mL (50 mg/0.1 mL). A dose of 50 mg/0.1 mL reconstituted intrastromal voriconazole is prepared just prior to administration.

5.3 Possible Side Effects of Study Medications

Natamycin: eye irritation, swelling, pain and/or redness, discharge

Voriconazole: eye irritation, swelling, pain and/or redness, discharge

Moxifloxacin: eye irritation, swelling, pain, or redness

Homatropine: eye irritation, swelling, pain, or redness; Eye dilation may result in temporary blurred vision, light sensitivity, and increased heart rate.

6. PROTECTION OF HUMAN SUBJECTS

6.1 Institutional Review Board Approval

6.1.1 Aravind Eye Hospital, Ethics Review Committee

The Ethics Committee will review the study protocol annually for ethical approval.

6.1.2 University of California, San Francisco Committee on Human Research (CHR)

The UCSF CHR will review the study protocol annually for ethical approval.

6.2 Informed Consent

Aravind personnel will obtain full written consent from each patient. The primary surgeon will screen participants and determine their eligibility. He/she will clearly explain the process and risks involved and will also ask the patient to sign any necessary consent documents. The study participant will have 7 visits total per eye included in the study over 3 months; visits include hospitalization for the first 7. The patient has the ability to withdraw at any time and will not be forced into anything with which he/she is not comfortable. Consent documents have been uploaded to the IRB application.

6.3 Risks to Study Participants

Fungal keratitis is a very serious sight threatening infection. The best therapy is still not known; therefore many treatments including topical and oral antifungals are often administered. Intrastromal Voriconazole injection, in addition to oral and topical antifungals, is current practice for the treatment of moderate to severe filamentous fungal corneal ulcers at Aravind Eye Hospital, Pondicherry. Participants in the study have the same risks as those undergoing treatment of their fungal keratitis who choose not to participate in the study.

It is not known whether or not the addition of oral antifungal is of benefit to these patients. The Mycotic Ulcer Treatment Trial II is an ongoing large prospective randomized controlled two arm clinical trial looking at whether or not oral voriconazole is of benefit in addition to topical antifungals. In this study we are asking patients to delay oral therapy for 3 days.

As with any surgery, there are risks associated with intrastromal injection which include risk of infection, or damage to the cornea, which could result in vision loss. There is a very small risk of full thickness perforation with the needle. If this were to occur, it would most likely be self-sealing, but may increase the risk of endophthalmitis, a severe sight threatening intraocular infection. Sterile precautions will be taken to ensure decreased risk of infection.

There may be some discomfort during follow-up testing (BSCVA, IOP, slit lamp, Pachymeter, Topography, and OCT testing and photo imaging of the eye), but this will be kept to a minimum. The participant will be asked to tell the doctor if any of this testing feels painful. There may be a medication reaction such as eye irritation, swelling, pain, redness, or discharge. If this occurs the risks of stopping the medication must be weighed against the severity of infection and other treatment options.

6.4 Privacy, Confidentiality and Data Security

All data will be stored and transferred using a secure server with de-identified information.

We will take steps to keep the participant's personal information confidential. Health information will be kept secure and separate from information which identifies participants. Upon enrollment, participants will be assigned a code that will be used instead of their name, medical record number or other personally identifying information. Electronic files for data analysis will contain only the participant code. The code will be stored at Aravind on a secure server and will only be accessed by study personnel – the PI, study coordinator, or other member of the study team. Access to study-related patient information will be limited only to members of the study team.

Only a small number of researchers related to this study will have direct access to the participant's medical information. Paper files will be stored in locked filing cabinets in restricted access offices at Aravind. Access to data/specimens is restricted to study personnel.

Information may be released to others outside of Aravind who are involved in coordinating or overseeing research, but information which identifies the participant will be kept secure. To ensure the success of this study, Aravind has partnered with University of California, San Francisco (UCSF)'s F.I. Proctor Foundation, which is a center that specializes in ophthalmology research. The coordinating center at UCSF will have access to de-identified information using a code number. UCSF's role is to support Aravind on the data analysis

portion of this study and assisting with the development of consent and study-related materials. UCSF will obtain IRB approval to take part in this study (IRB number: 15-17347).

6.5 Exclusion of Vulnerable Populations

The following populations will not be enrolled in this study: children (18 years and under), pregnant women, decisionally impaired adults, and prisoners.

6.6 Compensation to Participants

There is no additional cost or compensation for the study participant. There will be no compensation for research-related injury.

7. DATA COLLECTION AND MANAGEMENT

7.1 Data Collection and Entry

All data will be collected using hardcopy patient forms at Aravind during baseline and follow-up visits. De-identified data will be uploaded to a Dropbox account in order to share the data with the UCSF study coordinator. De-identified data will be input into a database using double data entry at UCSF.

7.2 Data Consistency, Validity, and Monitoring

Data monitoring reports will be prepared using STATA and Excel. If the forms are not filled out completely, the UCSF study coordinator will contact the person responsible for completing the form to provide the missing data, or clarify any inconsistent data. The Aravind study coordinator is the only person who is authorized to add missing data or make any changes to the study forms. All changes should be made with a red ink pen, and then signed and dated.

7.3 Data Storage and Security

De-identified data will then be input into a database using double data entry at UCSF. Data will be stored at UCSF for about 10 years.

8. STATISTICAL ANALYSIS PLAN

Please see the **Statistical Analysis Plan (SAP)** for details on the statistical analysis.

9. REFERENCES

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