Manual of Operating Procedures (MOP)

Repeated low-level red-light (RLRL) therapy Clinical Trial

Manual of Operating Procedures (MOP)

1. ELIGIBILITY/BASELINE EXAMINATION

To facilitate the recruitment process, study participants will be recruited through two pathways. The first pathway will be based on retrospective electronic medical record data within the past 12 months. Potential eligible children and their parents or guardians will be contacted and invited by a research assistant (RA). The second pathway will be through on-site recruitment via refraction or optometry clinic of the hospital, where participants diagnosed with myopia and their parents or guardians will be invited through posters, ads and physician referrals. The RA will post a marker on the medical records of these children to indicate their potential eligibility for the trial.

1.1. Informed consent

1. The principle investigator will review the informed consent form with children and their parents/guardians.

2. The principle investigator will then ask children and their parents/guardians if they have completely understood the content of the study and the informed consent form. Children and their parents/guardians will be given the opportunity to ask questions.

3. If children and their parents/guardians agree to participate in the eligibility test, the parents/guardians will be asked to sign off on the informed consent form, and the principal investigator will also sign off on the informed consent form.

1.2. Basic information questionnaire

Once the parents/guardians sign off on the informed consent form, the RA will allocate a study ID on the cover of a paper-based clinical record form (CRF). The RA will then ask for the participant's basic information, including demographics (name, date of birth, gender, ethnicity), date of examination and parent contact information (at least two), and record this information

in the CRF. The RA will ask the parents/guardians whether the participant has previously used atropine eye drops, orthokeratology, progressive addition lenses (PALs), or other methods to control myopia. A medical history of the child's eye disease, medication, surgeries and allergies will be recorded in the paper CRF.

1.3. Uncorrected Visual Acuity

An Early Treatment Diabetic Retinopathy Study (ETDRS) logMAR E chart (Precision Vision, Villa Park, Illinois, USA) with standard illumination will measure distance visual acuity at a distance of 4 meters (a reflecting mirror will be used to achieve this testing distance if the space is limited). This visual acuity chart will be placed at a height such that the line of 20/20 is the same as the height of the participant's eyes.

1. The examiner will turn on the light source of the lightbox and ask the participant to sit straight, avoid squinting, and not tilt back and forth or swing the body and/or head.

2. The examiner will explain to the participant the purpose and method of the visual acuity examination. Once the examination begins, the examiner will ask the participant to start from the top line and read each line at a time, and indicate the direction of the opening of the E optotype.

3. Each eye will be tested separately. When checking the right eye, the left eye will be covered by an occluding object; when checking the left eye, the object will occlude the right eye. The object should block the light as much as possible but avoid pressing the eyeball.

4. During the visual acuity examination, the participant will sit 4 meters away from the E chart, and start reading from the top row (6/60). If the participant wears glasses for distance, he/she will be asked to remove them for the examination.

5. If four of the five directions of the optotypes in the eye chart in the first row (20/200) can be correctly identified, the participant will be directed to read the fourth row (20/100). If there is still only one or fewer optotype directions that cannot be correctly identified, they will be asked to check the seventh row (20/50), followed by the tenth row (20/25), and finally the 11th row (20/20). At each level, if the direction of the four optotypes cannot be recognized, the participant will be directed to the previous line until at least four optotype directions are correctly recognized.

6. If the first line (20/200) of the optotype cannot be recognized correctly at a distance of 4 meters, the participant will be asked to stand 1 meter in front of the chart and repeat step 5. The examination will end at line 6 (20/63).

7. If the participant can identify at least four optotypes clearly in the sixth line, he/she will be asked to return to the position 4 meters away from the chart. The examiner will then retest whether the participant can see the optotypes of the first line (20/200) to confirm visual acuity.

8. If the patient cannot recognize any optotypes at 4m and 1m, the examiner will perform counting fingers, hand motions, manual, light positioning, and light perception examinations.

Result Record

Line-by-line examinations and methods for determining visual acuity will be conducted using the lowest line with the number of correctly identified optotypes ≥4/5 at 4 meters, or the lowest line with the number of optotypes ≥4/5 at 1 meter after conversion. For conversion, the denominator will be multiplied by 4, keeping the numerator unchanged. For example, if the correct identification of the optotype at 1 meter reaches the third line (20/125), the visual acuity is 20/(125×4), or 20/500.

1.4. Best-corrected visual acuity under non-cycloplegia and strabismus examination

1. First, autorefraction under non-cycloplegia (the specific procedures are the same as the autorefraction after cycloplegia; see step 1.7 below) will be performed. The best-corrected visual acuity of the child's left and right eyes will be recorded separately.

2. Cover-uncover test to confirm strabismus

The participant will be instructed to take off their glasses and open both eyes at the same time to look at an adjustment target. The participant will be tested with the target at 0.5 meters and 4 meters from the participant. A piece of black cardboard with a width of 5 cm and a length of 15 cm will be used as a cover plate, and the examiner will hold the cover plate and sit opposite the patient.

While covering the left eye first, the examiner will observe whether there is eye movement in the right eye when the cover plate is first removed. If there is eye movement, the right eye will have recorded strabismus. In addition, the direction of eye movement when uncovered will characterize the strabismus. If the eye moves from the medially to the laterally (temporally), it is esotropia; if it moves from the laterally to the medially (nasally), it is exotropia; and if it moves from a superior position back to a normal orientation, it is hypertropia. If the left eye is covered and there is no movement of the right eye, it indicates that the right eye has no tropia.

When uncovering, the examiner will observe the eye movement of the covered eye (left eye). If there is no eye movement in the left eye, it means that the left eye has no tropia; if the covered eye (left eye) has fixation movement from the oblique position to the straight position after removing the cover, it means that the left eye has a phoria. If the left eye stays in the oblique position after removing the cover, the eye (left eye) is only able to return to the straight position when the opposite eye (right eye) is covered, this indicates that the covered eye (left eye) has tropia.

If the participant has strabismus at 0.5 meters and 4 meters, the examiner will record them as having constant strabismus, and if there is strabismus at only one distance, it will be recorded as intermittent strabismus.

In the same way, the examiner will cover the right eye and observe the left eye and remove the cover of the right eye and observe the right eye.

3. The examiner will record the results of the best-corrected visual acuity under non-cycloplegia, whether there is strabismus and the type of strabismus on the paper CRF.

1.5. IOLMaster:

IOLMaster is an ocular biometric measurement instrument that uses the principle of partial coherence interferometry (PCI) to perform non-contact ocular biometric measurements, including axial length (AL), corneal radius of curvature (CC), anterior chamber depth (ACD), white-to-white (WTW) measurement and other parameters. AL is measured along the direction of the axis to obtain the distance of the optical path from the front surface of the cornea to the retinal pigment epithelium. Corneal curvature is measured by the distance between corneal reflections. ACD measurement is defined as the distance from the vertex of the anterior corneal surface to the anterior lens surface. WTW is determined by the iris.

Equipment

IOLMaster (Zeiss 500/XP)

Printer/paper

Pre-examination Preparation

1. Ambient lighting: The examination will be carried out in a bright environment;
2. Equipment calibration: The system will be calibrated every day using the model eye before commencing measurements;
3. Each day, the IOLMaster will be turned on;
4. Preparation: The participant will be directed to take a seat. If they wear glasses, they will be asked to remove them.

Operation Procedure

1. The IOLMaster will be turned on.
2. After 1 minute, the 'NEW PATIENT' template will appear. The examiner will use the 'Tab' key or the mouse to fill in the following details:

Surname: ***The surname of the participant***

First Name: ***The first name of the participant***

Date of Birth: ***(e.g.: 01/12/2009)***

ID: ***participant ID (e.g.: LLLT-100021)***

3. The participant will be asked to sit comfortably with the chin on the chin rest and the forehead against the forehead rest.

4. The examiner will adjust the chin rest to keep the child's eyes level with the red marks on both sides by twisting the handle on the examiner's left-hand side.

5. There are three ways to move the camera:

1)The height of the worktable can be adjusted using the 'up/down' button located on the upper left edge of the table.

2)Turning the joystick clockwise or counterclockwise can adjust the height of the camera.

3)The joystick can be moved forwards and backward and to both sides to adjust the position and focus.

6. Once the camera position is set and the focus is adjusted, a measurement can be taken by pressing the button on the top of the joystick.

7. Once the participant is in the correct position, he/she will see an amber light. They will be instructed to watch the light, and the examiner will adjust the focus such that the circle on the screen directly covers the pupil area. After aligning the center, the examiner will take a measurement by pressing the button on the joystick.

8. The light will then turn red and switch to axis length measurement mode (ALM). The participant will be asked to continue looking at the red light, and a single spot of white light will appear on the screen. The examiner can fine-tune the joystick forward and backward to obtain the best focus for the white spot of light. After completing this step, the examiner will press the button on the top of the joystick to take five continuous measurements. If the examiner finds it difficult to complete ALM or follow-up examinations, they will ask the participant to blink a few times prior to the examination.

[Note: The purpose of taking five measurements is to obtain the average of the measured values of the axial length. An **'Evaluation'** message indicates that there is a difference of more than 0.1 mm between a measured value and other values, and that the instrument cannot calculate the average value. To solve this, the inconsistent measurement values must be deleted

and taken again until the **'Evaluation'** information disappears, and then the average value of the eye axis length measurement is calculated. The examiner must ensure that the difference between the maximum and minimum values among the five values is less than or equal to 0.05mm.]

9. After obtaining five measurements, the examiner will press the space bar.

10. At this time, the participant will see the amber light (corneal curvature) again. A ring with a few dots inside will appear on the screen. The examiner will place these dots at the position of the pupil and fine-tune the focus to achieve a halo on each spot. The participant will be asked to blink before the measurement to maintain the integrity of the tear film and quickly complete the measurement. After completing this step, the examiner will press the joystick and take three measurements. A yellow circle on the screen indicates that the participant blinked during the measurement. In this case the measured value will be deleted, and the measurement will be retaken. After three measurements are obtained, the examiner will press the space bar. The error between the three measurements should not be more than 0.1mm.

11. The participant will be asked to continue looking at the amber light. A bright white light will be present in his/her peripheral visual field; the participant will be asked to continue looking at the amber light and ignore the white light (this measures the depth of the anterior chamber). At this time, two vertical lines will appear on the screen, representing the cornea and lens. The examiner will place the white light between the two vertical lines and press the button on the joystick. The examiner will ensure that the five measured values are equal in value.

12. The participant will be asked to continue looking at the amber light, and a ring with a few dots will appear on the screen. The examiner will place these dots on the pupil again and ask the patient to keep his/her eyes open wide and not to blink. The examiner will then press the joystick to take three measurements. The error between the three measurements should be not more than 0.1 mm.

At this point, the examiner will have completed examinations of one eye. The examiner will repeat these steps to examine the other eye. The IOLMaster will automatically move from the eye just checked to the other eye and begin again.

13. After completing all the examinations scheduled for the day, the examiner will ensure the equipment is shut shown by clicking the folder in the lower right corner of the screen, and then turning off the power.

14. The results of the participant will be recorded both in the paper-based CRF.

1.6. Cycloplegia

Equipment and materials

Topical ocular anesthetic eye drops (optional), 1% cyclopentolate, penlight, cotton swab

Pre-examination preparation

1. Ambient lighting: This test generally requires a dark room or dim lighting;

2. Preparation of the participant: The examiner will ask the patient if they have a history of drug allergy and inform them of precautions that should be taken after cycloplegia.

Examination procedure

1.The examiner will evaluate the peripheral anterior chamber depth of the tested eye, the iris pigment, and the light reflection of the pupil before the application of cycloplegia medications.

2.If appropriate, one drop of topical anesthetic will be applied to both eyes, the examiner and patient will wait 2 minutes.

3.The examiner will administer 1% cyclopentolate to both eyes twice - 1 drop for each eye, 5 minutes apart. The examiner will record the time of administering drugs.

4. The participant will be asked to close their eyes and rest for 20 minutes. After 20 minutes, the examiner will check the light reflex. If light reflex persists, the examiner will instill a third drop of 1% cyclopentolate and record the time.

5. After 15-20 minutes, the examiner will check the light reflex and pupil dilation of the patient with a torch. If the light reflex is absent and the pupil dilates to 6 mm or greater, cycloplegia has been reached.

Result Record

Based on the paper CRF and the on-site checklist, the examiner will record whether the participant achieved acceptable cycloplegia and the cycloplegic status. The information on the left and right eyes will be recorded separately. The categories of cycloplegic status are as follows:

1. The light reflex is absent; and the pupil diameter is >6mm; if this is the case, the participant is considered to have reached cycloplegia;
2. The light reflex is absent, and the pupil diameter is ⩽6mm;
3. The light reflex exists, and the pupil diameter is >6mm;
4. The light reflex exists, and the pupil diameter is ⩽6mm.

1.7. Autorefraction

Equipment Preparation: The examiner will use Topcon KR 8800 autorefractor to assess the participant's objective refraction after cycloplegia.

Examination Procedure

1. The examiner will press the power key of the refractor. The main screen will appear after a few seconds.
2. The examiner will enter the study ID on the paper-based CRF with a code scanner.
3. The participant will be asked to sit in front of the equipment and position head correctly.
4. The examiner will adjust the height of the equipment table and chair so that the participant is comfortable.
5. The examiner will then ask the participant to place his/her chin on the chin rest and let his/her forehead rest on the

forehead rest.

6. The examiner will adjust the height of the chin rest so that the participant's lateral canthus is at the same level as the eye height marking line.

7. The examiner will use the joystick to align positioning. The main body of the equipment can be fine-tuned horizontally or vertically by swinging the joystick in all directions. Alignment is achieved when the target eye appears in the center of the monitor screen.

8. When the main body of the refractor is moved forward, the equipment will focus on the target eye. A blurred spot of light will appear on the cornea. After focusing on the target eye, the spot of light will be at its smallest.

9. The examiner will fine-tune the main body of the refractor such that the smallest and brightest light spot appears in the calibration mark on the monitor screen.

10. The examiner will press the measurement switch. This will complete one measurement that will be displayed. The examiner will take three measurements for each eye. The examiner will ensure that desired precision (spherical and cylindrical power $\leq$ 0.25 D, axis $\leq$ 5 degrees) is achieved.

11. The results of the participant will be recorded both in the paper-based CRF.

1.8. Slit-lamp examination：

The paper-based CRF will be given to the slit-lamp examiner who will check the participant's eyelid, cornea, conjunctiva, anterior chamber, lens, and retina. The examiner will be asked to fill in the anterior and posterior segments slit lamp examination result (normal or abnormal; if there is an abnormality, the location of the abnormal and abnormalities will be stated) and sign off on the CRF.

1.9. OCT:

TOPCON DRI OCT Triton:

TOPCON Swept Source DRI OCT is a non-contact, high-resolution tomographic and biomicroscopic imaging device for in-vivo viewing and measurement of posterior ocular structures, such as choroidal thickness (ChT). ChT is measured as the perpendicular distance from the outer portion of the hyperreflective line corresponding to the retinal pigment epithelium to the posterior edge of the choroid as demarcated by the hyperreflective line corresponding to the choriosclera interface using built-in automated layer segmentation software. In addition, with the adoption of OCT Angiography, retinal and choroidal vascular features of the posterior eyeball are also measured.

Equipment

TOPCON DRI OCT Triton

Printer/paper

Pre-examination Preparation

1. Ambient lighting: This examination generally requires a dark room or dim lighting;
2. Each day, the TOPCON DRI OCT Triton will be turned on;
3. Equipment check: The system will be checked every day before commencing measurements;
4. Preparation: The participant will be directed to take a seat. If they wear glasses, they will be asked to remove them.

Operation Procedure

1. The TOPCON DRI OCT Triton will be turned on.
2. After 1 minute, the Patient Screen will appear on the computer interface. The examiner will select the “New Patient” tab and fill in the following details with the keyboard:

Last Name:	***The last name of the participant***
First Name:	***The first name of the participant***
Date of Birth:	***(e.g.: 01/12/2009)***
Gender:	***Male or Female***
Patient ID:	***participant ID (e.g.: LLLT-100021)***

3. The examiner will first peel off one chin rest paper and clean forehead rest with an alcohol pad, and then ask the participant to place his/her chin on the chin rest and let his/her forehead rest on the forehead rest comfortably. The examiner will adjust the height of the chin rest by chinrest up/down button on the left of control panel so that the participant's lateral canthus is level with the eye height marking line.

4. The examiner will select **"Radial Dia. 9.0mm × 12 overlay 16"** on "**MACULAR**" capture icon in the Menu interface by touching the screen with fingers on the touch display. Then the examiner will touch Capture and get into the image capture interface.

5. As the internal fixation target turns on, the participant will be instructed to look at the green light (fixation target) in the center with the right eye.

6. The examiner will move the instrument body in right and left / up and down directions with the joystick until the participant's right eye gets in the center of the fundus or anterior segment live image area. Then, the examiner will bring the ( ) scale toward the patient's pupil, and make sure that the pupil is larger than the ( ) scale. The base of the instrument will be pulled slowly toward the participant side, and the fundus image appears on the fundus live image area.

7. Two alignment bright spots for the working distance alignment and the split lines for the focal distance alignment become visible on the fundus live image area. The examiner will move the joystick back and forth until the two bright spots are changed to one spot, and operate the joystick up and down, right and left to bring the spot into the ( ) scale. The split lines will automatically change into one line when the autofocus function of the instrument is turned on.

8. The examiner will adjust the illumination level to make the photograph at an appropriate brightness.

9. The examiner will tell the patient that he is about to take a picture of his/her right eye and ask he/she not to blink and to keep watching the green light (fixation target).

10. After making sure that the alignment bright spot and split line are correctly positioned on the touch display. The examiner will press the photography button on the top of the joystick, and the OCT photograph is automatically obtained.

11. The examiner will check the OCT photograph in the preview screen and the image quality score is required to be greater than 60.

12. The participant is instructed to have a short relaxation after each capture, but the chinrest position is maintained while the examiner reviews the scan.

13. The examiner will repeat steps 5-12 until the image quality meets the requirement.

14. For **"3D Macular 6.0×6.0mm"** and **"3D Macular Angio 7.0×7.0mm"** scan mode, the examiner will touch the Menu button and select the corresponding capture icons and repeat steps 5-13 to complete the right eye examination.

15. After obtaining the right eye OCT and OCTA scans, the participant will be instructed to maintain the chinrest and forehead rest position, and the examiner will move the base towards the participant's left side. Steps 4-14 will be repeated in the left eye examination.

16. When the session is completed, the participant is instructed to relax and move away from the chinrest.

17. The data will be automatically saved in the built-in software.

18. The images of the participant will be stored with file name including patient name, ID number, gender, type of scan, date of exam, eye examined, type of report, and date of report. The examiner will record the completion condition in the paper-based CRF.

1.10. Formal enrollment and randomization:

1.The RA will determine whether the screened participant meets the inclusion criteria given below:

1) Inclusion criteria

1) Provision of consent

2) Age: ⩾8 and ⩽ 13 years at enrolment

3) Myopia: Spherical equivalent refractions (SERs) under cycloplegia: -1.00 to -5.00 diopters (D)

4) Astigmatism of 2.50 D or less

5) Anisometropia of 1.50 D or less

6) Corrected monocular logMAR visual acuity (VA): 1.0 or better

7) Consent to participate in the random allocation of grouping

8) Fluent in English

9) Willing and able to participate in all required activities of the study

10) Ethnically African, Hispanic or Caucasian

2) Exclusion criteria

1) Strabismus and binocular vision abnormalities in either eye

2) Ocular abnormalities in either eye or other systemic abnormalities that affect participation in all required activities of the study.

3) Prior treatment of myopia control in either eye including but not limited to drugs, orthokeratology, progressive addition lenses, bifocal lenses, etc.

4) Other reasons including but not limited to severe physical and cognitive disability, that the physician may consider inappropriate for enrolment

5) Noncompliance with treatment

6) Children whose parents do not sign the informed consent

2. Only participants who fully meet the inclusion criteria and do not meet any of the exclusion criteria will be included in the study. The RA will register the results in paper CRF during the screening period and enroll children who fully meet the inclusion criteria.

3. After baseline data collection, participants will be equally randomized to one of the two groups: the intervention group (repeated low-level red light therapy + single vision spectacles) and the control group (single vision spectacles), following simple randomization. The randomization will be computer-generated by a statistician who will not have any active involvement in the study (group A will be the intervention or group B will be the control). The allocation ratio will set at 1:1 to ensure balance among the two study groups. The randomization assignment will be generated randomly, numbered consecutively, and will be kept in a sealed, opaque envelope. Assignment will be revealed to RA only after participants complete their baseline visits. The RA will explain to the participant and parents/guardians the relevant requirements after enrollment, following the protocol for the group the participant belongs to.

4. Participants in the intervention group will be given a red-light therapy device and shown the video explaining the use of the device to ensure that it will be used correctly. The treatment time will be emphasized again: five days per week, twice a day, three minutes per session, with a minimum four-hour interval between treatments. For participants in the control group, the examiner will emphasize follow-up time and explain that single-vision glasses for myopia can be worn.

2. FOLLOW-UP EXAMINATION

2.1: Appointment

Before the follow-up window (+/-1 week), the receptionist at the ACO will remind the participant's parents/guardians by telephone of the follow-up appointment. An appointment will be made, and the participant's parents/guardians will be informed of the examination location.

2.2：Questionnaire

The RA will administer questionnaires to the participants' parents and themselves to obtain feedback following treatment.

2.3. Uncorrected visual acuity examination (same as baseline)

2.4. Best-corrected visual acuity under non-cycloplegic refraction (same as baseline)

2.5. IOLMaster (same as baseline)

2.6. Cycloplegia (same as baseline)

2.7. Auto-refraction (same as baseline)

2.8. Slit-lamp examination (same as baseline)

2.9. OCT

The operation procedures and scanning mode selection are the same as the baseline. Additionally, the examiner needs to press **"Follow up"** button on the top right of the Menu interface in the touch display and the button will change to **"Follow up ON"** (orange). In this case, the OCT system will read the baseline photographed data that is relevant to the entered patient ID and the selected photography icon to decide the same scan position.

3. Handling of Withdraws

A study investigator will withdraw and stop study treatment the participation in the study for the following reasons:

1) Any severe adverse event (a sudden loss of vision > two lines, or a scotoma developed in the centre of the visual field).

2) Any evidence indicates annual refraction progression $\geq$ 1.50 Diopters.

3) The participant meets an exclusion criterion (either newly developed or not previously identified) that precludes further study participation.

4) Any other reason in the Investigator's opinion.

5) Safety information becomes available to stop the study.

6) The total compliance with the treatment is less than 20%.

Participants are free to withdraw from participation in the study at any time upon request. Participants who withdraw from the study will be surveyed for reasons for withdrawal. Permission to utilize the data collected before a withdrawal will be requested. If they decline, all data of participants who withdraw from the study will be deleted from the record system.