Excelencia en oftálmicos

Protocol:

SOPH231-1221/I

Title: Phase I clinical study, to evaluate the safety and tolerability of PRO-231 ophthalmic solution, versus VIGAMOXI,® on the ocular surface of ophthalmologically and clinically healthy subjects.

Information about the molecule under study
Generic name: Moxifloxacin 0.5%
Distinctive name: PRO-23 1
Indication: Conjuntivitis bacteriana.

Protocol Information
Study Phase: I
Version: 2.0
Release Date: 27-jul.-22

This protocol has been carried out in accordance with the principles of the Declaration of Helsinki and will be carried out in accordance with Good Clinical Practices and in compliance with the ICH guidelines and current local legislation.

Sponsor: Laboratorios Sophia, S.A. de C.V.

Change History

The following changes were made to generate version 2.0 of the protocol, dated Jul 27-22 (replaces version 1.0 dated Feb 18-22).

- Updated version number and date in the corresponding sections and in the header.
- Added operational definition of hypotheses in the Summary*, 3.2* Hypotheses sections. The wording was improved and information regarding the statistical methodology was updated in the sections *Summary, 10.3 Statistical methodology* and in section *10.3.1 Analysis of populations.* In section *7.4 Outcome variables*, in section *7.4.3 Definition of variables*, the statistical test "Student's t-test of independent groups" is added for the variable *incidence of expected AEs and related to the interventions (excluding conjunctival hyperemia and/or chemosis) (bis)* included in *Table 2. Operational definition of variables.*
- Information is added regarding the adverse reactions reported with moxifloxacin ophthalmic solution 5mg/mL, classified by apparatus and systems, as well as by their criterion of frequency of presentation.
- Annex *16.5 LogMAR Fraction Conversion Table* was amended in section *16. Annexes.*
- Spelling corrections were made, writing improvements were made, and bibliographic citations were updated that correspond to the modifications made throughout the document.

Content

Table and Figure Index

Responsible for the study

The administrative structure of the sponsoring party, corresponding to Laboratorios Sophia, S.A. de C.V., is shown in Table 1. Responsible for the study

Function§	Name/ Contact	Affiliation ¥
Medical Head of the Study	Dr. Leopoldo Martín Baiza Durán leopoldo.baiza@sophia.com.mx	Regional Director of Medical Affairs
Study Director	Dr. Oscar Olvera Montaño oscar.olvera@sophia.com.mx	Regional Medical Affairs Manager
Author of the Protocol	Dra. Gisela García Sánchez gisela.garcia@sophia.com.mx	Specialist in Therapeutic Area
Biostatistics	Dr. Patricia del Carmen Muñoz Villegas patricia.munoz@sophia.com.mx	Head of Biostatistics
Operational Manager	M. en C. Miriam Isabel Serrano Andrade miriam.serrano@sophia.com.mx	Regional Manager of Clinical Research

¥ Employees of Laboratorios Sophia, S.A. de C.V Av. Paseo del Norte No.5255, Col. Guadalajara Technology Park, Carretera Guadalajara-Nogales Km13.5 C.P. 45010 Zapopan, Jalisco, Mexico Tel +52(33) 3000 4200
§ In the event of staff turnover, the medical head of the study will be in charge of appointing the interim manager.

***Board 1.** Responsible for the study*

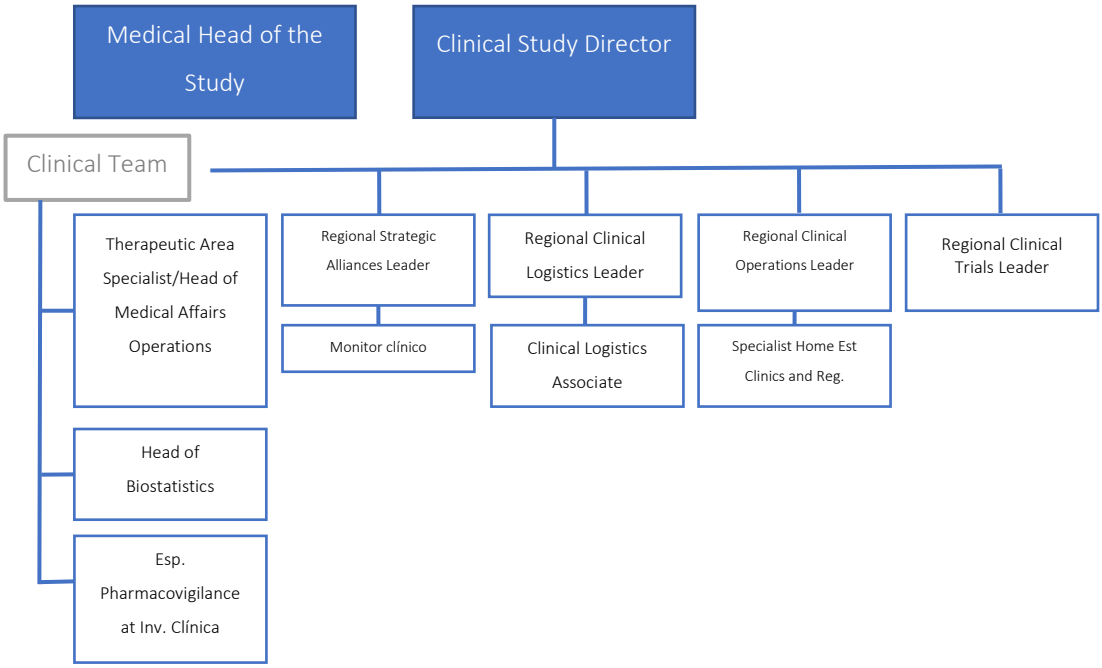


Figure 1. Administrative structure.

Signature Page

From the sponsor

Name: Dr. Leopoldo Martín Baiza Durán	**Signature**
Title: Medical Head of the Study	**Date**

Name: Dr. Oscar Olvera Montaño	**Signature**
Title: Study Director	**Date**

Name: M. en C. Miriam Isabel Serrano Andrade	**Signature**
Title: Operational Manager	**Date**

Name: Dra. Gisela García Sánchez	**Signature**
Title: Protocol Author	**Date**

Investigator Agreement

I agree to conduct this clinical study according to the design and guidelines of this protocol, in accordance with its provisions. I declare that I will conduct the study in accordance with the standards of Good Clinical Practice, and I will report all information or data in accordance with the protocol, in particular, any adverse event. I will also handle clinical supplies, provided by the sponsor, strictly in accordance with this protocol. I understand that the information that identifies me may be used by the sponsor. Because the information contained in this protocol and the Investigator's Manual is confidential, I understand that it is prohibited from sharing it with any third party, which is not involved in the approval, supervision, or conduct of the study. I will ensure that I take the necessary precautions to protect the information from loss, inadvertent disclosure, or access by unauthorized third parties.

Name: *[Insert full name of researcher]* **Title:** Principal Investigator **Name of the center:** [*Insert Name of Study Site]* **Geographic location (city/state/country)** *[Enter the geographical data of the center]*	**Signature** **Date**

List of abbreviations

A	Alpha
°C	Celsius degrees
DNA	Deoxyribonucleic acid
AV	Visual acuity
AVMC	Best-corrected visual acuity
BPC	Good clinical practice
CBP	How much is enough to
CDM	*Clinical Data Management*
CIS	Research Ethics Committee
CI	Research Committee
CIOMS	Council for International Medical Sciences Organizations (ICE) en inglés, *Council for International Organizations of Medical Sciences)*
COFEPRIS	Federal Commission for the Protection against Sanitary Risks
CONSORT	Consolidated *Standards of Reporting Trials*
IUD	Intrauterine device
EA	Adverse Event/Adverse Events
eCRF	Electronic *case report form*
FCI	Informed consent form
H0	Null hypothesis
H1	Alternate hypothesis
ICH	International *Council for Harmonisation*
ICMJE	*International Committee of Medical Journal Editors*
ICO	Eye Comfort Index
IP	Principal Investigator
ITT	By *intention to treat*
LogMAR	*Logarithm of the minimum angle of resolution*
Ml	Milliliters
Mmhg	Millimeters of mercury
NOM	Official Mexican Standard
OD	Right eye (Latin for its acronym *oculus dexter)*

WHO	World Health Organization
YOU	Left eye (Latin for *oculus sinister)*
PAE	Statistical analysis plan
PI	Research Products
PIO	Intraocular pressure
PP	Population by protocol
RAM	Adverse drug reaction
RNEC	National Clinical Trials Registry
S-W	Saphiro-Wilk test
SCE	Foreign body sensation
SDV	Source *document verification*
SPSS	Statistical *package for the social sciences*
SRAM	Suspected adverse drug reaction
TEA	Evaluation time
TF	Fluorescein staining
TID	Three times a day (by its Latin acronym *ter in die)*
UFTLS	Pharmacovigilance and Technovigilance Unit of Sophia Laboratories
USED	United *States of America*
x2	Chi-square

Página intencionalmente dejada en blanco

1. Protocol Summary

1.1 Synopsis

<table>
<tr><td colspan="2">Sponsor Name:
Laboratorios Sophia, S.A. de C.V.</td></tr>
<tr><td colspan="2">Product Name:
PRO-231</td></tr>
<tr><td colspan="2">Active ingredient name:
Moxifloxacino hydrochloride</td></tr>
<tr><td colspan="2">Title of the study:
Phase I clinical study, to evaluate the safety and tolerability of PRO-231 ophthalmic solution, versus VIGAMOXI,® on the ocular surface of ophthalmologically and clinically healthy subjects.</td></tr>
<tr><td>Study Number:
SOPH231-1 2 21/I</td><td>Date of creation:
Dec 1st-21</td></tr>
<tr><td>Protocol version:
2.0</td><td>Release Date:
27-Jul-22</td></tr>
<tr><td>Therapeutic indication:
Ocular antibiotic.</td><td>Use:
Conjuntivitis bacteriana</td></tr>
<tr><td>Estimated duration of the study (from the first patient's first visit to the preparation of the final report):
5 to 6 months</td><td>Clinical Development Phase:
I</td></tr>
<tr><td colspan="2">Objectives:

<u>Main objective:</u>

• To evaluate the safety and tolerability of the PRO-231 formulation, versus VIGAMOXI,® on the ocular surface of ophthalmologically and clinically healthy subjects.

<u>Specific objectives:</u>

• Primary:</td></tr>
</table>

- To assess the safety of PRO-231 ophthalmic solution applied to the ocular surface, in healthy volunteers, versus VIGAMOXI,® by:
  - To compare the incidence of adverse events (AEs), unexpected, related to interventions.
  - Incidence of conjunctival hyperemia and/or chemosis between interventions.
  - Changes in best-corrected visual acuity (MCVA) assessed with the Snellen chart between interventions.
  - Changes in ocular surface integrity using fluorescein staining, using the Oxford scale between interventions.
- To evaluate the tolerability of PRO-231 ophthalmic solution applied to the ocular surface, in healthy volunteers, versus VIGAMOXI,® by:
  - Changes in Eye Comfort Index (JI) score between interventions.

- *Side:*
  - To assess the safety of PRO-231 ophthalmic solution applied to the ocular surface, in healthy volunteers, versus VIGAMOXI,® by:
    - Incidence of expected and intervention-related AEs (excluding conjunctival hyperemia and/or chemosis).
  - To evaluate the tolerability of PRO-231 ophthalmic solution applied to the ocular surface, in healthy volunteers, versus VIGAMOXI,® by:
    - Presence of ocular symptoms (burning, foreign body sensation, pruritus and tearing) between interventions.

Hypothesis:

H0= PRO-231 ophthalmic solution has a safety and tolerability profile similar to that of VIGAMOXI® in clinically healthy subjects.

$$H_0: ST_A = ST_B \; o \; ST_A - ST_B = 0$$

H1= PRO-231 ophthalmic solution does not have a similar safety and tolerability profile as VIGAMOXI® in clinically healthy subjects.

$$H_1: ST_A \neq ST_B \; o \; ST_A - ST_B \neq 0$$

Operational definition of similarity: To reject H0, the p-value in the primary variables must be ≤ 0.05 and the 95% CI of this difference must not include zero. Both conditions for hypothesis testing (p-value and 95% CI) must be met, otherwise the result will be interpreted as **"absence of evidence",** not **"evidence of absence".**

Study Design:

Phase I clinical study, controlled, comparative, parallel-group, single-blind, single-center.

Number of subjects (planned and analyzed):

Number of planned subjects: 38 (19 subjects per arm).

Diagnosis and main inclusion criteria:

Ophthalmologically and clinically healthy subjects.

Selection criteria:

Inclusion criteria:

- Have the ability to voluntarily give their signed informed consent.
- Ophthalmologically and clinically healthy subjects.
- Be able and willing to comply with scheduled visits, treatment plan, and other study procedures.
- Age 18 to 45 years.
- Both sexes.
- Women of childbearing potential must ensure the continuation (initiated ≥ 30 days prior to signing the informed consent form [ICF]) of the use of a hormonal contraceptive method or intrauterine device (IUD) during the study period.
- AVMC of 20/30 or better in both eyes.
- Corneal staining ≤ grade I on the Oxford Scale.
- Have an intraocular pressure ≥ 10 and ≤ 21 mmHg.

Exclusion criteria:

- History of hypersensitivity to fluoroquinolones or any of the components of the PI.
- Be a user of topical ophthalmic medications of any pharmacological group.
- Be a user of medicines by any other means of administration.
- History of eye surgery in the last 6 months.
- Wearing contact lenses for less than two weeks prior to the start of the study, and during the study intervention period.
- For women: being pregnant, breastfeeding, or planning to become pregnant within the study period.
- Have participated in any investigational clinical study 30 days prior to inclusion in this study.
- Have previously participated in this same study.
- History of any chronic-degenerative disease, including Diabetes Mellitus or Systemic Arterial Hypertension.
- Known diagnosis of liver or heart disease.
- Have active inflammatory or infectious disease at the time of study entry.

- Have unresolved injuries or trauma at the time of study entry.
- Having undergone surgical, non-ophthalmologic procedures within the past 3 months.
- Have received doses of vaccine against the Influenza virus or Coronavirus, 7 days prior to the inclusion of this study, or plan to receive it during the study intervention.
- Be or have an immediate family member (e.g., spouse, parent/legal guardian, sibling, or child) who is employed by the research site or sponsor, and who is directly involved in this study.
- Positive smoking (specified as cigarette smoking regardless of quantity and frequency, 4 weeks prior to study inclusion and during the study intervention period).
- Positive alcoholism (specified as the consumption of alcoholic beverages, regardless of quantity and frequency, 72 hours prior to the inclusion of the study and during the study intervention period).

Elimination criteria:

- Withdrawal of the informed consent form.
- Presentation of serious adverse event, whether or not related to the interventions, that, in the judgment of the Principal Investigator (PI) and/or sponsor, could affect the patient's ability to safely continue with study procedures.
- Non-tolerability or hypersensitivity to any of the compounds used during the tests (fluorescein, tetracaine).
- No tolerability or hypersensitivity to any of the investigational drugs.
- Adherence < 80% determined by the subject's diary and corroborated by the final weight of the research products (PI) with respect to the initial weight.

Duration of treatment:	Duration of the subject in the study:
7 days	13 days

Interventions:

- Investigational product, dosage, and route of administration:
  - PRO- 231 . Moxifloxacin 0.5% Ophthalmic Solution. Laboratorios Sophia, S.A. de C.V. Zapopan, Jalisco, Mexico.
  - Dosage: 1 drop every 6 hours (3 daily applications [TID], in the right eye [OD]).
  - Route of administration: Topical ophthalmic.

- Comparator product, dosage and route of administration:
  - VIGAMOXI ® Moxifloxacino 0.5% Solución oftálmica. Alcon Laboratories, Inc., Fort Worth, Texas, USA.
  - Dosage: 1 drop every 6 hours (TID) in OD.
  - Route of administration: Topical ophthalmic.

Evaluation criteria:

Primary outcome variables:

Safety

- Incidence of Intervention-Related Unexpected AEs (Time to Assessment [ET]: Days 3, 8, and 12)
- Incidence of conjunctival hyperemia and chemosis. (TE: day 3 and 8)
- Changes in the AVMC evaluated with the Snellen chart. (TE: day 3 and 8)
- Changes in ocular surface integrity using fluorescein staining, using the Oxford scale. (TE: day 3 and 8).

Tolerability

- ICO score changes. (TEE: day 8)

Secondary outcome variables:

Safety:

- Incidence of expected and intervention-related AEs (excluding conjunctival hyperemia and/or chemosis). (TE: day 3, 8 and 12)

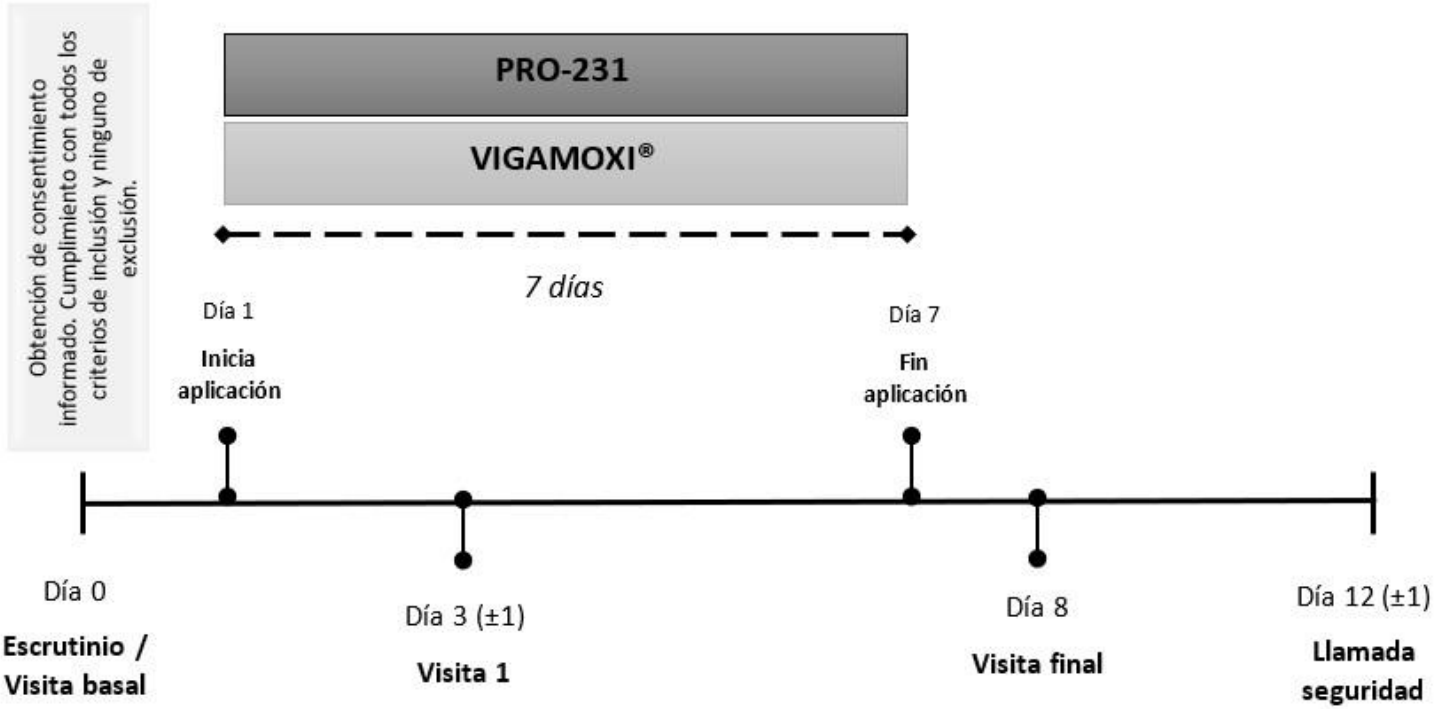
Tolerability:

- Presence of eye symptoms (burning, foreign body sensation, pruritus and tearing). (TE: day 3 and 8)

Statistical methodology

The analysis of the data collected in the study will be carried out by means of specialized statistical software (SPSS available version or R software). The data will be expressed with measures of central tendency: mean, standard deviation and/or ranges for quantitative variables, and frequencies and/or percentages for qualitative variables. The Shapiro-Wilk test (S-W) will be used to determine if the distribution is normal in the data obtained in each study group ($p>0.05$). If the normality in the distribution of the data is verified, to rule out differences between groups, the Student's t-statistic will be used for independent groups, while intragroup differences will be analyzed with the Student's t-test for repeated measures. In case of $p<0.05$ in S-W, the statistical analysis to rule out differences between groups will be performed by means of the Mann-Whitney U test for quantitative variables, while intragroup differences will be performed by Wilcoxon's rank test. The difference between the qualitative variables will be analyzed by means of the X2 statistic (Chi-squared) or Fisher's exact (for expected values less than 5). Dichotomous variables will be analyzed by the McNemar test when applicable. An alpha (α) ≤ 0.05 will be considered as statistical significance, in addition to this, the 95% CI will be calculated for these differences. For primary outcome variables, H0 will be rejected, when $p \leq 0.05$ and the 95% CI does not include zero.

1.2 Study diagram



1.3 Subject Timeline

Procedures	Scrutiny / Basal Visit Day 0	Visit 1 Day 3 ± 1	Final Visit Day 8	Call Security Day 12 ± 1
Signing the informed consent form	X			
Comprehensive medical history	X			
Ophthalmological medical history	X			
Urine pregnancy test	X		X	
Eligibility Criteria	X			
Eye symptoms	X	X	X	
AVMC Measurement	X	X	X	
Previous Segment Evaluation	X	X	X	
Fluorescein staining evaluation	X	X	X	
Intraocular pressure measurement	X	X	X	
Posterior Segment Evaluation	X		X	
Subject and IP code assignment	X			
Delivery of the PIs and indicate the start of application the next day (day 1)	X			
Return of IP			X	
Adherence assessment		X	X	
Evaluation of adverse events	X	X	X	X
Eye Comfort Index	X		X	
Subject Diary Delivery	X			
Return / Evaluation of the subject's Journal			X	
Subject continuity assessment		X		

Abbreviations: AVMC, best corrected visual acuity; PI, research products.

2. Introduction and background

2.1 Theoretical framework

Humans are exposed to a variety of microorganisms, including bacteria, viruses, and fungi. In most cases, these microorganisms do not cause infection thanks to the external barriers (skin and mucous membranes) that protect us from their invasion, and the immune system. However, some microorganisms can directly invade these external barriers or be introduced into the body through surgical or traumatic wounds; Additionally, the patient's immune system may be compromised, allowing microorganisms, which do not normally cause any problems, to generate an infection. [1]

Various compounds have been used to help the immune system fight pathogenic microorganisms. An important property of anti-infective drugs is their selective toxicity, eliminating microorganisms while causing minimal or no adverse reactions to the host. [1]

These drugs are not usually active against all species of microorganisms in the same category; The species against which the drug shows intrinsic activity is called *Spectrum of activity* of the drug. Narrow-spectrum drugs are only effective against a few species of microorganisms, while broad-spectrum drugs have activity against a wide variety of species. At the same time, microorganisms can develop resistance mechanisms against drugs to which they were originally susceptible, limiting the effectiveness of anti-infective drugs. [1]

Bacteria are the largest contributor to eye infections in the world and can affect different eye structures causing conjunctivitis, keratitis, endophthalmitis, blepharitis, orbital cellulitis and dacryocystitis. [2, 3]

Bacterial conjunctivitis is one of the most common eye infections and is estimated to account for up to 1% of consultations at the first level of care. It is characterized by eye discomfort, conjunctival injection, and mucopurulent discharge, and has a significant economic and social impact, as a result of disruption of the patient's normal activities, as well as absenteeism from work and/or school. It is usually self-limiting (duration of 1 to 2 weeks); however, empirical treatment with broad-spectrum anti-infective drugs is recommended to shorten the course of the disease, improve patient comfort, decrease transmissibility, promote early return to work or school, and reduce the risk of serious complications that may compromise vision [1, 2, 3, 4, 5, 6, 7]

The pathogens most commonly implicated in bacterial conjunctivitis include strains of *Staphylococcus aureus*, *Streptococcus pneumonie*, *Streptococcus viridians*, *Haemophilus influenzae*, *Serratia marcescens* and *Pseudomona aeruginosa*. [8, 9]

Although broad-spectrum topical antibiotics are usually used empirically, several factors need to be considered when selecting a treatment, such as how quickly it kills the pathogen causing the infection, how well it penetrates eye tissues, and the potential impact on the development of bacterial resistance. [9]

Moxifloxacin is a broad-spectrum antimicrobial that belongs to the fourth generation fluoroquinolones and thanks to its 8-methoxy group, it has improved activity against strains of *Streptococcus* and *Staphylococcus*, as well as moderate to excellent activity against gram-negative organisms that cause ocular pathologies. [6]

Moxifloxacin is a dose-dependent bactericide. Like the other fluoroquinolones, it interferes with the bacterial life cycle by binding to DNA gyrase (topoisomerase II) and topoisomerase IV. Both are essential enzymes for the replication, translation, repair, and recombination of bacterial deoxyribonucleic acid (DNA). Human cells lack these enzymes, so they are not affected by fluoroquinolones.[6, 8, 9, 10] [1, 8]

The antimicrobial activity or spectrum of fluoroquinolones will depend on their class, due to the variation in affinity and binding strength to topoisomerases, as well as to the bacterial species. [10]

They are generally well-tolerated drugs with a low incidence of adverse reactions after systemic administration. When they occur, these reactions are usually mild and reversible, the most common being: gastrointestinal (nausea, vomiting and diarrhea), dermatological (rash, urticaria, pruritus) and central nervous system (headache). However, they have also been associated with serious adverse events, including *Clostridium difficile,* prolongation of the QT interval, tendinitis, dysglycemia, liver toxicity, phototoxicity, acute renal failure and seizures. [1, 8, 11, 12]

The frequency of adverse events with topical fluoroquinolone administration is low; The most frequent ocular adverse reactions reported after the application of the 0.5% moxifloxacin ophthalmic solution are: decreased visual acuity, dry eye, keratitis, eye discomfort, ocular hyperemia, eye pain, pruritus, subconjunctival hemorrhage, tearing and burning. The most frequent non-ocular adverse events reported are: dysgeusia, fever, cough, otitis media, pharyngitis, rash and rhinitis. . [1][13, 14]

The following adverse reactions have been reported during clinical studies with Moxifloxacin 5 mg/mL ophthalmic solution and are classified according to the following criteria: very common, occurring in 1 or more in 10 patients (≥1/10); common, occurring in 1 or more in 100 patients (≥1/100 to <1/10); rare, occur in 1 or more in 1,000 patients (≥1/1,000 to <1/100); rare, occurring in 1 or more in 10,000 patients (≥1/10,000 to <1/1,000); very rare, there is less than one case in every 10,000 patients (<1/10,000); or unknown frequency (a number cannot be estimated from the available data). Adverse reactions are ordered by organ or system affected, from the most severe to the mildest: [15, 16, 17]

Classification by organs and systems	Frequency	Adverse Reactions [MedDRA Preferred Terms (v.15.1)]
Blood and lymphatic system disorders	Strange	Decreased hemoglobin
Nervous system disorders	Rare	Headache
	Strange	Paresthesia (tingling or numbing sensation)
Eye disorders	Common	Eye pain, eye irritation

	Rare	Dry eye, conjunctival hemorrhage, red eye, itchy eyes, eyelid edema (swelling of the eyelids), eye discomfort.
	Strange	Corneal alteration, conjunctivitis, blepharitis (inflammation of the edge of the eyelids), conjunctival edema, blurred vision, decreased visual acuity, asthenopia (visual fatigue), redness of the eyelids.
Respiratory disorders	Strange	Nasal discomfort, pharyngolaryngeal pain (sore throat), foreign body sensation in the throat
Gastrointestinal disorders	Rare	Dysgeusia (bad taste in the mouth)
	Strange	Vomit

Additional adverse reactions identified in post-marketing surveillance include the following: frequency cannot be estimated from available data. Within each classification by organ and system, adverse reactions are presented from the most serious to the mildest. [15, 16, 17]

Classification by organs and systems	Adverse reactions [MedDRA preferred terms (v.15.1)]
Immune system disorders	Hypersensitivity (allergic reactions)
Nervous system disorders	Seasickness
Eye disorders	Corneal ulcers, increased tearing, photophobia (sensitivity to light), eye discharge.
Heart disorders	Palpitations
Respiratory disorders	Dyspnea (shortness of breath)
Gastrointestinal disorders	Nausea
Skin disorders	Erythema (redness of the skin), purito (itching), rash (hives or blisters on the skin), hives (reddish hives that may cause itchy skin)

Available ophthalmic fluoroquinolones are indicated for the treatment of bacterial conjunctivitis and keratitis, with a treatment regimen of one drop three times daily for 7 days (the total duration of therapy will depend on the severity of the clinical picture and the course of the infection). In addition, they have been used off-label as prophylaxis in surgical procedures and ocular trauma to prevent endophthalmitis. However, because new molecules such as gatifloxacin and moxifloxacin have a higher spectrum and lower resistance, they should be reserved for the treatment of more severe eye infections.[8, 9, 15, 16] [17, 18] [17, 19]

There is no antimicrobial that provides ideal coverage for all pathogens at any site of infection. However, moxifloxacin 0.5% ophthalmic solution is a unique, preservative-free antibiotic (thanks to

its concentration it does not require the addition of preservatives to the formula), which offers broad-spectrum activity, and a moderate to high clinical success rate against common ocular pathogens. In addition to this, thanks to its concentration and its high penetrance to the ocular tissues, it allows a dosing regimen at infrequent intervals, simplifying its administration and making it more convenient for patients. [6, 7, 9, 10]

2.2 Background on the investigation

2.2.1 From the research question

Is the application of PRO-231 ophthalmic solution safe and tolerable in clinically healthy subjects?

The importance of the question that precedes this paragraph is that Laboratorios Sophia S.A. de C.V. does not have clinical studies with products identical to the PRO-231 solution, although there are with similar products (different fluoroquinolones and excipients).

2.3 Problem statement

Bacterial conjunctivitis is one of the most common eye infections and it is estimated that it represents up to 1% of consultations at the first level of care, generating a significant economic and social impact, as a result of the interruption of the patient's normal activities, as well as absenteeism from work and/or school. Although it is usually a self-limiting condition, with an average duration of 1 to 2 weeks, empirical treatment with broad-spectrum anti-infective drugs is recommended to shorten the course of the disease, improve patient comfort, decrease transmissibility, promote early return to work or school, and reduce the risk of serious complications that may compromise vision [9][1, 2, 3, 4, 5, 6, 7]

Fluoroquinolones are antibiotics whose usefulness in the area of ophthalmology has already been proven, as they have been used for decades as a treatment and/or prophylaxis for eye infections. Its effectiveness and safety have given this family of drugs a significant place within the therapeutic options of today's ophthalmologist; however, despite being generally well-tolerated drugs with low toxicity, it was decided to compare PRO-231 with VIGAMOXI,® the topical formulation of moxifloxacin 0.5% currently marketed in our country, to verify that its safety and toxicity profile is equal to the latter.

2.4 Rationale for the study

Although eye infections can be considered minor infections, in some cases, they can become complicated and leave irreversible sequelae that can compromise the patient's vision. For this reason it is important to prescribe topical antibiotics for the treatment of eye infections, or as prophylaxis in surgical procedures and eye trauma. An important property of anti-infective drugs is their selective

toxicity, eliminating microorganisms while causing minimal or no adverse reactions to the host. [17, 18] [1]

Moxifloxacin hydrochloride, the active ingredient in PRO-231 ophthalmic solution, is a fourth-generation fluoroquinolone, which has already been approved for use in the treatment of bacterial conjunctivitis and keratitis caused by susceptible strains; and it is already marketed by international ophthalmic product laboratories. It has the advantage of being preservative-free, and in addition, offers broad-spectrum activity, and a moderate to high clinical success rate against common ocular pathogens. In addition to this, thanks to its concentration and its high penetrance to the ocular tissues, it allows a dosing regimen at infrequent intervals, simplifying its administration and making it more convenient for patients. [6, 7, 9, 10]

In our country, this antibiotic formulation is marketed and approved under the name VIGAMOXI® (Alcon Laboratories, Inc. Fort Worth, Texas, USA) for the treatment of bacterial conjunctivitis. Clinical studies are required to evaluate that the safety and tolerability of PRO-231, prepared by Laboratorios Sophia S.A. de C.V., is comparable to that of VIGAMOXI,® and which are the basis of future clinical studies that prove its efficacy. The current commitment of Laboratorios Sophia, S.A. de C.V. is to generate its own information for its products that serves for the pertinent regulatory procedures, and as a scientific basis for their commercialization.

3. Objectives and hypotheses

3.1 Objectives

3.1.1 Main objective:

To evaluate the safety and tolerability of the formulation PRO-231 versus VIGAMOXI,® on the ocular surface of ophthalmologically and clinically healthy subjects.

3.1.2 Specific objectives:

3.1.2.1 Primary

- To assess the safety of PRO-231 ophthalmic solution applied to the ocular surface, in healthy volunteers, versus VIGAMOXI,® by:
    - To compare the incidence of unexpected AEs related to interventions.
    - Incidence of conjunctival hyperemia and/or chemosis between interventions.
    - Changes in the AVMC, evaluated with the Snellen chart, between interventions.
    - Changes in ocular surface integrity using fluorescein staining, using the Oxford scale between interventions.
- To evaluate the tolerability of PRO-231 ophthalmic solution applied to the ocular surface, in healthy volunteers, versus VIGAMOXI,® by:
    - Changes in Eye Comfort Index (JI) score between interventions.

3.1.2.2 Secondary

- To assess the safety of PRO-231 ophthalmic solution applied to the ocular surface, in healthy volunteers, versus VIGAMOXI,® by:
    - Incidence of expected and intervention-related AEs (excluding conjunctival hyperemia and/or chemosis).
- To evaluate the tolerability of PRO-231 ophthalmic solution applied to the ocular surface, in healthy volunteers, versus VIGAMOXI,® by:
    - Presence of ocular symptoms (burning, foreign body sensation, pruritus and tearing) between interventions.

3.2 Study hypothesis

H0: PRO-231 ophthalmic solution has a similar safety and tolerability profile as VIGAMOXI® in clinically healthy subjects.

$$H_0: ST_A = ST_B \; o \; ST_A - ST_B = 0$$

H1: PRO-231 ophthalmic solution does not have a similar safety and tolerability profile as VIGAMOXI® in clinically healthy subjects.

$$H_1: ST_A \neq ST_B \; o \; ST_A - ST_B \neq 0$$

Operational definition of similarity: To reject H0, the p-value in the primary variables must be ≤ 0.05 and the 95% CI of this difference must not include zero. Both conditions for hypothesis testing (p-value and 95% CI) must be met, otherwise the result will be interpreted as **"absence of evidence",** not as **"evidence of absence".**

4. Study design

4.1 Study Overview

Phase I clinical study, controlled, comparative, parallel-group, single-blind, single-center.

4.2 Rationale for the study design

The design of the study (clinical trial) is considered the highest standard of data quality when seeking to explore the effect of an intervention. The drug development phase (phase I) corresponds to the objective of the study, which is to evaluate safety and tolerability, so the intervention time is short and the sample size required is smaller than that of an efficacy clinical trial.

Although this is a comparative study with two intervention groups, only the principal investigator and the person responsible for the statistical analysis will be blinded, because the primary packaging of the comparator cannot be modified to resemble the product under investigation. Despite this, the secondary packaging for the products will be the same and the researcher will not know the allocation.

4.3 Expected duration of the study

The total duration of the study, from the first subject's first visit to the preparation of the final report, is estimated to be 5 to 6 months.

4.4 Duration of the subject in the study/treatment

The approximate duration of each subject in the study is up to 13 days.

5. Study population

5.1 Eligibility Criteria

5.1.1 Inclusion criteria

- Have the ability to voluntarily give their signed informed consent.
- Ophthalmologically and clinically healthy subjects.
- Be able and willing to comply with scheduled visits, treatment plan, and other study procedures.
- Age 18 to 45 years.
- Both sexes.
- Women of childbearing potential must ensure continued use (initiated ≥ 30 days prior to signing the CRF) of the use of a hormonal contraceptive method or IUD during the study period.
- AVMC of 20/30 or better in both eyes.
- Corneal staining ≤ grade I on the Oxford Scale.
- Have an intraocular pressure ≥ 10 and ≤ 21 mmHg.

5.1.2 Exclusion criteria:

- History of hypersensitivity to fluoroquinolones or any of the components of the PI.
- Be a user of topical ophthalmic medications of any pharmacological group.
- Be a user of medicines by any other means of administration.
- History of eye surgery in the last 6 months.
- Wearing contact lenses for less than two weeks prior to the start of the study, and during the study intervention period.
- For women: being pregnant, breastfeeding, or planning to become pregnant within the study period.
- Have participated in any investigational clinical study 30 days prior to inclusion in this study.
- Have previously participated in this same study.
- History of any chronic-degenerative disease, including Diabetes Mellitus or Systemic Arterial Hypertension.
- Known diagnosis of liver or heart disease.
- Have active inflammatory or infectious disease at the time of study entry.
- Have unresolved injuries or trauma at the time of study entry.
- Having undergone surgical, non-ophthalmologic procedures within the past 3 months.
- Have received doses of vaccine against the Influenza virus or Coronavirus, 7 days prior to the inclusion of this study, or plan to receive it during the intervention of the same.

- Be or have an immediate family member (e.g., spouse, parent/legal guardian, sibling, or child) who is employed by the research site or sponsor, and who is directly involved in this study.
- Positive smoking (specified as cigarette smoking regardless of quantity and frequency, 4 weeks prior to study inclusion and during the study intervention period).
- Positive alcoholism (specified as the consumption of alcoholic beverages, regardless of quantity and frequency, 72 hours prior to the inclusion of the study and during the study intervention period).

5.2 Criteria for elimination and/or substitution of subjects

5.2.1 Elimination Criteria

- Withdrawal of the consent form under information.
- Presentation of a serious adverse event, whether or not related to the PIs, that, in the judgment of the PI and/or the sponsor, could affect the patient's ability to continue with study procedures safely.
- Non-tolerability or hypersensitivity to any of the compounds used during the tests (fluorescein, tetracaine).
- No tolerability or hypersensitivity to any of the investigational drugs.
- Adherence < 80% determined by the subject's diary and corroborated by the final weight of the PI with respect to the initial weight.

5.2.2 Substitution of subjects

The sponsor may decide to replace subjects who withdraw their FCI or those who have loss of follow-up, until the necessary sample size is completed.

5.3 Counting failures

A screening failure is defined as those participants who agree to participate in the study, giving their consent, but who are not assigned to the treatment group, i.e., do not enter the study. It is necessary to report at least the following information on counting failures:

- Demographics.
- Details of the failure to count (specify whether it is due to eligibility criteria, which one, or some other reason for the failure).
- Presence of serious adverse events during the count.

This is necessary to comply with the CONSORT (*Consolidated Standards of Reporting Trials*) guidelines for the publication of results or to respond to possible questions from regulatory authorities.

5.4 Recruitment and retention strategies

The duration of the subject's participation in the study is approximately 13 days, during which time the subject will be required to attend three visits in total and will be given a safety call, which corresponds to the baseline visits, visit 1, final visit and safety call. Strategies to improve subject retention include, but are not limited to:

- Clearly inform the objectives of the study.
- Make calls or send text messages to remind you of appointments or activities to do.
- Provide a printed calendar and an identification card in order to remember appointments and activities that will be carried out, in addition to the estimated time of their duration.
- Systematic organization of the study procedures, so that the subject does not last longer than necessary in his visit.
- Minimize subject wait times.

All materials to be delivered to the subject or recruitment strategies implemented by the center will be submitted for approval by the corresponding committees.

5.5 Procedure in case of loss of follow-up

For this protocol, loss of follow-up is defined as those subjects who were assigned the PI, who at some point were active subjects of the study, but their final evaluation could not be completed.

In the event that the subject does not terminate their participation due to withdrawal of consent or major deviation, the last visit, in which their withdrawal was determined, will be considered their final visit. Subjects who are removed due to the presence of AEs will continue with the follow-up that is defined until the closure of their AE.

In cases where the participating subject does not attend their appointment, the research center must make a call to find out the reason and will try to make a new appointment within the established window period or an unscheduled appointment. In the event that it is not possible to make an appointment, the subject will be considered as loss of follow-up and will be asked about the presence of adverse events and the reason for leaving the study, as minimum data.

A loss of follow-up <20% is considered not to be a problem for the validity of the results obtained. [20, 21]

5.6 Identification of the subject

Study subjects will be identified by a number and the initials of their name.

The initials of the study subject will be obtained starting with the first letter of the name, followed by the first letter of the first surname and the first letter of the second surname, obtaining a maximum of three letters, in case the person has two names or compound surname, the first letter will always be used.

Example:

To. Arieh Daniel Mercado Carrizalez — to. Initials: AMC

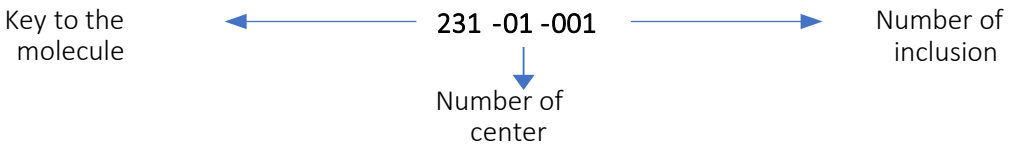
B. Juan De la Torre Orozco — b. Initials: JDO

In the counting stage, the participant number will be assigned consecutively, using 3 consecutive digits.

Once the subject has been selected, they will be assigned a number with which they will be identified throughout the study. This code will be made up of eight numbers in the following order from left to right:

- three digits of the molecule under study according to the name by the sponsor.
- Two digits corresponding to the research centre number.
- three digits of the number following their inclusion assigned in the research centre.

Example of assigned number:



6. Investigational product and treatment

6.1 Interventions

6.1.1 Investigational product

- PRO- 23 1 . Moxifloxacin 0.5% Ophthalmic solution . . Zapopan, Jalisco, Mexico. Laboratorios Sophia, S.A. de C. V
- Route of administration: Ophthalmic.
- Pharmaceutical form: Ophthalmic solution.
- Presentation: multi-dose dropper bottle, 5 mL.
- Prepared by: Laboratorios Sophia, S.A. de C.V.

- Solution description: transparent, free of visible particles and yellow in color.
- Packaging description: White bottle made of low-density polyethylene filled to 5 mL.

6.1.2 Investigational Product Dosage

Dosage: 1 drop three times a day (DID) for 7 days, in the right eye (OD).

6.1.3 Treatment with the investigational product

The product under investigation will be delivered at the conclusion of the baseline visit. The subject under investigation will begin the application the next day (day 1) by administering one drop every 6 hours, applying a total of one drop three times a day to the right eye during the 7 days of application.

6.1.4 Comparison Product

- VIGAMOXI ® Moxifloxacino 0.5% Ophthalmic solution . Alcon Laboratories, Inc. Fort Worth, Texas, USA.
- Route of administration: Ophthalmic.
- Pharmaceutical form: Ophthalmic solution.
- Presentation: multi-dose dropper bottle, 5 mL.
- Prepared by: Alcon Laboratories, Inc.
- Description of the solution: transparent, greenish-yellow solution.
- Packaging description: Natural low-density polyethylene bottle and a dosing cap with polypropylene closure (Drop-trainer® dispensing system) filled to 5 mL.

6.1.5 Comparator Product Dosage

Dosage: 1 drop TID for 7 days, in OD.

6.1.6 Treatment with the comparator product

The product under investigation will be delivered at the conclusion of the baseline visit. The subject under investigation will begin the application the next day (day 1) by administering one drop every 6 hours, applying a total of one drop three times a day to the right eye during the 7 days of application.

6.2 Storage and handling of the investigational product in the study center

The delivery will be made by means of a courier service contracted by the sponsor, expressly selected for this purpose, to the address of the research center according to the study plan.

The reception will be carried out by the assigned personnel of the research team. You will need to check that the primary packaging (box) is in good condition. In the event that you show alterations or defects in its integrity that in your judgment could have damaged the content, you must report it to the sponsor. If the package does not show significant defects, it will proceed to open it.

Inside the shipment you must locate the acknowledgment document and the temperature meter (*data logger*). You must check that the temperature recorded complies with what is specified for transport and safekeeping. It will verify the content (PI) with what is reported in the document. In case the document corresponds to the content, you will sign the receipt and send it to the sponsor. Otherwise, it will notify the sponsor.

Storage and safeguarding is the responsibility of the research center. The medication must be stored in a secure area with restricted access.

The storage temperature should be 2° to 25°C.

From its receipt at the center and until there is no stock of PI stored, the research center has the obligation to review the storage conditions of the PI on a daily basis and manually record, in the designated format, the temperature set by the *data logger* (current, minimum and maximum temperature). Such data will be reviewed by the clinical monitor during their monitoring visits according to the records stored in the *data logger's memory.*

In the event of loss of material, it must be documented in the logbook of inputs and outputs along with a clear description of the mechanism by which the loss occurred.

Upon completion of the protocol, all study material will be retrieved by the sponsor as part of the closing visit. The final delivery of material will be made by the principal investigator or the person designated by him to deliver material at the end of the study.

The sponsor reserves the right to initiate civil and criminal action against the principal investigator in the event of a lack of undocumented material at the conclusion of the study.

6.3 Concomitant treatments and medications (permitted and prohibited)

Any medication that is used, in addition to appearing in the clinical note, must be registered in the concomitant medication section of the eCRF (*electronic Case Report Form*).

6.3.1 Permitted Medications

- Ophthalmic:

All permitted medications applied via ophthalmic medication during the study must wait a minimum period of 10 minutes from the last application of the treatments under study or reference. The above in order to avoid the interaction of treatments in the tear film, based on the flow index and physiological tear volume. [22]

  - Tetracaína 0.5%
  - Tropicamide 0.8% /Phenylephrine 5%
  - Hipromelosa 2%
  - Fluorescein

- Systemic:

In case participants of childbearing potential decide to use a hormonal contraceptive method, the following will be allowed:

  - Oral contraceptives.
  - Subdermal implant.
  - Parenteral injection.
  - HORMONAL DIU.

6.3.2 Prohibited medicines:

- Any ophthalmic or systemic medications that are not on the list of allowed medications.

6.4 Procedure for monitoring and measuring adherence

For more than four decades, numerous investigations have been conducted on the appropriate way to measure and quantify medication adherence, however, none has reached a consensus to stand as the gold standard, both in cross-sectional and longitudinal studies. [23, 24, 25, 26, 27, 28, 29, 30]

There are different procedures to measure adherence to pharmacological interventions. The most common procedure includes self-reports, which include: interviews with the subject, questionnaires and self-monitoring diaries. Its strengths are speed, flexibility, low cost and ease of implementation; They have a high degree of specificity for non-adherence, however, the sensitivity and reliability for adherence is low. [30, 31]

The biochemical measurement of the drug, or its metabolite, is one of the methods that best confirms the use of the drug. However, in addition to raising costs and being impractical, it is of little use in the context of ophthalmic applications, since concentrations at the peripheral level could be undetectable; and samples of other tissues involve more invasive methods that would not be advisable. [30]

There is no standardized parameter to define adequate adherence, it must be defined and delineated by the objectives of the particular research. [30]

For this study, a minimum adherence of 80% (per subject's diary) will be considered to be necessary to meet the objectives of the research; In turn, the information in the diary will be corroborated with the final weight of the research product with respect to its initial weight. Therefore, subjects with adherence of less than 80% will not be considered for the study.

6.5 Strategies to improve adherence

1. The PI will sensitize the subject to the importance, in order to achieve the objectives of the study, of the correct application of the concomitant treatment.
2. Direct questioning by the PI about the application of the concomitant treatment.
3. Delivery of a printed calendar specifying the date of the visit and its activities.

If deemed necessary, text messages may be sent as reminders. The content of these messages must be previously approved by the Research Ethics Committee (ERC).

7. Study methods and procedures

7.1 Research centre

This study will be carried out in a research center previously evaluated by the sponsor. The center will be an institution or establishment where health research is carried out that complies with current regulations.

The research center will be responsible for forming a multidisciplinary research team to execute the clinical study according to the protocol. It is their prerogative to design the organization and select the personnel who will perform the functions. However, it is necessary for the sponsor that the PI and sub-investigator be ophthalmology specialists.

Any person who is designated, under the responsibility of the PI, a part of the study monitoring (sub-investigator, nurse, etc.) or a specific function of participation in the study (pharmacist, administrative assistant, study coordinator, etc.) must appear in the "Delegation of Responsibilities".

The competence and training of any person who has direct participation in the activities of the study must be verified prior to the performance of any activity related to the protocol. The above must be recorded and the documents that constitute evidence of this competence and/or training must be kept in the master file of the study. The competence and training of the personnel who have functions in the study, both at the central level and in the study center, is the responsibility of the principal investigator. [32]

The sponsor must ensure that all study site personnel participating in the study are adequately trained on the study (research protocol, investigator's manual, amendments, standard operating procedures, etc.) and on the Good Clinical Practices of the ICH (International Council on Harmonization) *International Council for Harmonisation*), before the start of their participation in it. Training must be recorded in writing and those records must be filed in the master record of the study. [32]

7.2 Clinical Study Registration

This clinical study will be registered by the sponsor in public clinical trial registries before its start (inclusion of the first subject): National Registry of Clinical Trials (RNEC) of the Federal Commission for the Protection against Sanitary Risks (COFEPRIS) and in a primary registry platform of the World Health Organization (WHO). WHO primary registries meet specific criteria for content, quality and validity, accessibility, unique identification, technical capacity, and administration. WHO Primary Registries meet the requirements of the International Committee of Medical Journal Editors (ICMJE).

7.3 Assignment of treatment

The assignment/randomization of the treatment will be carried out centrally using a validated electronic system from an external provider, previously evaluated and authorized by Laboratorios Sophia S.A. de C.V. There are two treatment groups. Participants will be randomized in a 1:1 ratio to treatment with PRO-231 or VIGAMOXI.® Having the same probability of being assigned to one or the other treatment. After signing the FCI, the subject will receive a participant number with which all their information will be encrypted pseudonymity during collection and completely anonymized during analysis. The generation will be carried out by a third party, authorized by Laboratorios Sophia, S.A. de C.V., through its electronic system, through a list of random numbers generated by software (SAS Institute Inc, Cary, NC, USA). The information pertaining to this third party will be on file. Only the principal investigator and the person responsible for the statistical analysis will be blinded, because the primary packaging of the comparator cannot be modified to resemble the product under investigation. Despite this, the secondary packaging for the products will be the same and the researcher will not know the allocation. IP will be identified by means of labels in accordance with current and applicable regulations, which must contain at least:

- Sponsor's name, address, and phone number.
- Dosage form and route of administration.
- Number of doses.
- Batch number.
- Legend "Exclusively for clinical studies".
- Expiration date.

7.4 Outcome variables

7.4.1 Primary outcome variables

7.4.1.1 Security

- Incidence of unexpected AEs related to interventions.
- Incidence of conjunctival hyperemia and chemosis.
- Changes in the AVMC evaluated with the Snellen chart.
- Changes in ocular surface integrity using fluorescein staining, using the Oxford scale.

7.4.1.2 Tolerability

- ICO score changes.

7.4.2 Secondary outcome variables

7.4.2.1 Security

- Incidence of expected and intervention-related AEs (excluding conjunctival hyperemia and/or chemosis).

7.4.2.2 Tolerability

- Presence of eye symptoms (burning, foreign body sensation, pruritus and tearing).

7.4.3 Definition of variables, methods and scales for their measurement

Board 2. Operational definition of variables

Variable	Conceptual Definition	Operational Definition	Measurement Type	Reference value	Statistical test
Incidence of Intervention-Related Unexpected AEs	Any adverse medical event that occurs in the clinical research subject to whom any of the interventions were administered and that has a certain coincidental relationship to the interventions [33]	All AEs (according to protocol) in which the PI suspects a causal relationship with the interventions will be reported	• Discrete quantitative • Nominal qualitative	• Relationship with IP • Severity • Causality	• Student's t-test of independent groups • U de Mann Whitney* • *Pearson X2* or Fisher Exact*
Incidence of conjunctival hyperemia	It is the reaction of the conjunctiva to a stimulus, a red appearance secondary to the vasodilation of the conjunctiva vessels of variable intensity is seen	Direct observation. Classification by Efron scale. *See attachment* 16.2. Efron Scale for Conjunctival Hyperemia	• Ordinal qualitative	Degrees: 0 = Normal, I = Muy live, II = Mild, III = Moderate and IV = Severe	• *Pearson X2* or Fisher Exact* • McNemar Test*
Incidence of Chemosis	It is defined as conjunctival edema, the result of an inflammatory reaction. It is classified as present or absent	The evaluator will use a narrow beam of light at 60° and will measure whether the conjunctiva separates ≥ 1/3 of the total	• Nominal qualitative	Present / Absent	• *Pearson X2* or Fisher Exact* • McNemar Test*

Variable	Conceptual Definition	Operational Definition	Measurement Type	Reference value	Statistical test
		eyelid opening or if it exceeds the gray line			
Changes in the AVMC	Visual spatial acuity is the ability to distinguish separate elements of an object and identify them as a whole. It is quantified as the minimum angle of separation (located at the nodal point of the eye) between two objects that allows them to be perceived as separate objects	Cartilla de Snellen. Ver anexo 16.5 Fraction to LogMAR conversion table.	• Continuous quantitative	20/30 (0.2 LogMAR) or better	• Student's t-test of independent groups • U de Mann Whitney*
Changes in the integrity of the ocular surface	Detection of corneal and conjunctival epithelial defects by means of the CT using the Oxford scale	Direct observation with slit lamp, graduation on Oxford scale. See attachment 16.3 Oxford scale	• Ordinal qualitative	Degrees: The TF is presented in a series of panels (A – E). The staining points vary from 0 – 5 for each panel and from 0 – 15 for the total exposed area of the conjunctiva and cornea	• *Pearson X2* or Fisher Exact* • McNemar Test*
ICO score changes	The ICO is a questionnaire designed to measure irritation of the ocular surface, it assesses symptoms focused on the comfort associated with ocular surface alterations. Elevated values indicate more severe symptoms	The evaluator will apply the questionnaire to the subject and allow the subject to answer it calmly without any pressure and/or coercion. See annex 16.4 Eye Comfort Index (JI)	• Continuous quantitative	0 – 100	• Student's t-test of independent groups • U de Mann Whitney*

Variable	Conceptual Definition	Operational Definition	Measurement Type	Reference value	Statistical test
Incidence of expected and intervention-related AEs (excluding conjunctival hyperemia and/or chemosis). (bis)	Any adverse medical event that occurs in the clinical research subject who was administered any of the interventions and that has a casual relationship to them [33]	All intervention-related AEs will be reported according to the protocol.	• Discrete quantitative • Nominal qualitative	• Incidence • Severity • Causality	• Independent group student's t-test • U de Mann Whitney* • *Pearson X2* or Fisher Exact*
Incidence of eye symptoms (burning, SCE, pruritus, and tearing)	**Burning:** refers to the sensation of stinging or eye irritation. **SCE:** refers to the sensation of "sand" inside the eye. **Pruritus:** This refers to the itching or tickling sensation caused by the need to rub or scratch. **Tearing:** It refers to excessive tear production [34, 35, 36, 37]	The evaluator will perform the interrogation to rule out or confirm the general presence of symptoms	• Nominal qualitative	Present / Absent	• *Pearson X2* or Fisher Exact* • McNemar Test*

Abbreviations: AVMC, best corrected visual acuity; AE, adverse event; ICO, eye comfort index; IP; principal investigator; SCE, foreign body sensation; TF, fluorescein staining; X2, Chi-square. *When applicable.

7.4.4 Description of the variables, methods and scales for their measurement

For clinical purposes, ophthalmological evaluations will be carried out in both eyes, and their record will be recorded in the clinical file. However, in order to obtain results and comply with the assumption of independence within the variables, only the examinations and qualifications of the right eye will be recorded in the eCRF when appropriate. Next, the description of the variables is made, which are not in order of execution.

7.4.4.1 Conjunctival hyperemia

It is defined as the simplest reaction of the conjunctiva to a stimulus, with a red appearance secondary to vasodilation of the conjunctival vessels of variable intensity. It will be graduated using the Efron scale. See annex [38]16.2. Efron Scale for Conjunctival Hyperemia

The PI will record in the file and the eCRF the grade, in grades (Grade 0 = normal, grade 1 = traces, grade 2 = mild, grade 3 = moderate, grade 4 = severe), granted for conjunctival hyperemia OD and OS respectively.

Management as AE: An adverse event will be considered when the grade is greater than or equal to grade 3 (moderate).

7.4.4.2 Chemosis

It is defined as conjunctival edema, the result of an inflammatory reaction. It is qualified as present or absent. The evaluator will use a narrow beam of light at 60° and will measure whether the conjunctiva separates from the sclera by ≥ 1/3 of the total eyelid opening or if it exceeds the gray line. [39]

The PI will record in the file and the eCRF if the chemosis is absent or present.

Management as AE: An adverse event will be considered when present.

7.4.4.3 Ocular symptomatology

The subject will be questioned directly about the presence in general (since the last visit) of the following symptoms: burning, foreign body sensation, pruritus, tearing.

7.4.4.3.1 Ardor

Feeling of irritation or stinging in the eyes. [37]

7.4.4.3.2 Foreign Body Sensation

Feeling of sand inside the eye. [36]

7.4.4.3.3 Pruritus

It is an itching or tickling sensation that causes the need to rub or scratch the eye. [34]

7.4.4.3.4 Lagrimeo

Excessive tear production or flow. [35, 36]

The subject will respond only if the symptom is absent or present

These symptoms will be analyzed as one of the variables of the study and will not be considered an adverse event.

7.4.4.4 Best-corrected visual acuity

Visual acuity (VA) is a test of visual function. Visual spatial acuity is the ability to distinguish separate elements of an object and identify them as a whole. It is quantified as the minimum angle of separation (located at the nodal point of the eye) between two objects that allows them to be perceived as separate objects.

Snellen's notation is described as the distance at which the test is performed, divided by the distance at which the letter is vertically equivalent to 5 arc minutes. Thus, at 6 meters a letter 6/6 (20/20) is equivalent to 5 minutes of arc, a letter 6/12 (20/40) is equivalent to 10 minutes, and a letter 6/60 (20/200) is equivalent to 50 minutes. The Snellen fraction can also be expressed as a decimal place (i.e., 20/20 = 1 and 20/40 = 0.5). [40]

VA will be evaluated at baseline, without refractive correction with the Snellen chart (see annex 16.1 Cartilla de Snellen). It will be located in a place with adequate lighting (natural or artificial) and at a distance of 3m from the subject to be evaluated. Visual acuity of the right eye (OD) will be taken initially, asking the subject to hold both eyes open and using an occluder to cover the contralateral eye; the subject will read aloud the lines that the evaluator points out, the line of smaller letters that he can see will be noted by the evaluator in fraction as the AV of OD in the clinical record and the eCRF, then the same evaluation will be performed in the left eye and its record only in the clinical file.

Next, the subject's best refractive correction (obtained by autokeratorrefractometer and subjective tests) will be evaluated and the examination will be repeated using the refraction obtained. This result will be reported as best-corrected visual acuity.

Both data (AV and AVMC) will be reported for statistical purposes in logarithmic figures (LogMAR) in the eCRF.

A fraction conversion table is attached to LogMAR. (see annex 16.5 Fraction to LogMAR conversion table.)

Management as AE: A decrease in 2 or more lines of sight in BCVA compared to that obtained at baseline will be considered an adverse event.

7.4.4.5 Ocular surface staining

Fluorescein (TF) staining: A drop of topical anesthetic will be instilled in the bottom of the conjunctival sac, then a second drop will be applied to the tip of the fluorescein strip, allowing it to sit on the strip for 5 seconds to elute the dye, shaking off the excess at the end. A small contact of the strip is made with the conjunctiva at the fundus of the temporal sac, while the subject looks upwards, without damaging the conjunctiva. Between 4 and 8 minutes after the instillation of the staining, the assessment of the fluorescein stain in the cornea is carried out, with the slit lamp with a cobalt blue filter. It will be graded according to the Oxford Scale. See attachment [41, 42]16.3 Oxford scale

The PI will record in the file and the eCRF the grade, in grades (Grade 0 = Absent, grade I = Minimal, grade II = Mild, grade III = Moderate, grade IV = Marked, grade V = Severe), granted for the corneal staining of OD and OS respectively.

Management as AE: An adverse event will be considered when the rating is greater than or equal to grade III (moderate).

7.4.4.6 Adverse events

As described in the 8. Evaluation and management of adverse events An adverse event is defined as any unfavorable medical occurrence in a subject to whom an investigational product is administered, regardless of causal attribution.

Adverse event management shall be carried out as described in section 8. Evaluation and management of adverse events and incidents.

The Principal Investigator will record in the corresponding section of the eCRF the adverse events that the study subjects may present in addition to referring them in the clinical record.

For an adequate evaluation of adverse events, in addition to directed questioning, it is necessary to perform a comprehensive ophthalmological evaluation at each visit, which consists of: ophthalmological examination of the eyelids and adnexa; anterior and posterior segment that is performed in a routine ophthalmological check-up, whose procedures are not specifically included in the study variables. Posterior pole evaluation can be with direct or indirect ophthalmoscopy, with or without pharmacological mydriasis, at the discretion of the PI. An assessment of the fundus will be carried out in search of abnormalities that alter the result of the study. IOP will be measured in this evaluation, with the Goldmann tonometer, it should be measured after the evaluation of stains. The result of the assessment will be recorded in the clinical file. In the eCRF, only findings that are considered so by the Principal Investigator will be reported as an adverse event.

The ocular adverse events that can occur with PRO-231, according to what is reported in the literature, are: ocular burning, dysgeusia after instillation, foreign body sensation, ocular pruritus, conjunctival hyperemia, chemosis, photophobia and whitish corneal precipitates. The frequency of systemic adverse events with ophthalmic fluoroquinolone administration is low.[1, 13] [1]

7.4.4.7 Eye Comfort Index

It is a questionnaire designed to measure ocular surface irritation with Rasch analysis to produce estimates on a linear interval scale (ratings: 0-100). Similar to the index for ocular surface diseases, the ocular comfort index (ICO) assesses symptoms. The ICO contains 8 items (one positive and eight negative) that focus on discomfort associated with ocular surface alterations. Each of these questions has two parts, which separately inquire about the frequency and severity of symptoms. [43]See attachment 16.4 Eye Comfort Index (JI)

At the baseline visit and final visit, the evaluator will deliver the questionnaire to the subject and allow the subject to answer it calmly without any pressure and/or coercion, only assisting him if he has difficulty understanding any of the questions.

As it is a subjective measurement scale according to the subject's symptomatology, variations in the ICO cannot be managed as AEs.

7.5 Description of procedures or assessments during the study

The different procedures that will be done during the study are described below. The list may not be in order, and it may be arranged in the most optimal way according to the needs of the research center.

7.5.1 Signing of informed consent

Procedure by which it is guaranteed that the subject under investigation has voluntarily expressed his intention to participate in this research, after having understood the information that has been given to him about the objectives of the research, benefits, discomforts, and possible risks.

7.5.2 Preparation of medical history (includes ophthalmological and general medical history)

It must be carried out in accordance with the provisions of NOM-004-SSA3-2012 of the clinical file. Considering that the clinical record is the unique set of information and personal data of a subject, which is integrated into any type of establishment for medical care, whether public, social or private, which consists of written, graphic, imaging, electronic, magnetic, electromagnetic, optical, magneto-optical and any other type of documents, in which, Health personnel must make the records, annotations, where appropriate, certificates and certifications corresponding to their intervention in the subject's medical care, in accordance with the applicable legal provisions. [44]

And it will consist of:

- Medical history: It must be prepared by medical personnel and other professionals in the health area, in accordance with the specific information needs of each of them in particular, it must have, in the order indicated, the following sections:
    - Interrogation. – It must have at least: identification form, if applicable, ethnic group, hereditary-family history, pathological and non-pathological personal history, current condition (inquire about previous conventional, alternative and traditional treatments) and interrogation by devices and systems (especially ophthalmological).
    - Physical exam. – You must have at least: vital signs (temperature, blood pressure, heart and respiratory rate), weight and height (when applicable).
    - Previous and current results of laboratory, cabinet and other studies (when applicable).
    - Diagnoses or clinical problems (when applicable).
    - Prognosis (when applicable).

- Therapeutic indication (when applicable).

7.5.3 Eligibility Criteria

It is the assessment that the subject meets all the inclusion criteria and none of the exclusion or elimination criteria.

7.5.4 Assigning Subject Code

It is the granting of a code that represents the subject in the study. This code is assigned when the research subject is included in the study.

7.5.5 Adverse events

Described in the 8. Evaluation and management of adverse events

7.5.6 Vital Signs Measurement

It is the measurement of respiratory rate, heart rate and blood pressure. These measurements can be made with a stethoscope and mercury or digital sphygmomanometer.

7.5.7 Urine Pregnancy Test

The pregnancy test will be performed at the baseline visit and at the final visit. The Researcher must provide the research subject with a female, of reproductive age (there is no natural or induced menopause, defined as 12 consecutive months of amenorrhea). Women of childbearing potential with contraceptive methods including bilateral tubal obstruction should take a pregnancy test. To perform the test, the subject will be allowed to go to the bathroom, have privacy for the performance of the test, and after the test is performed, the Investigator must corroborate the result by observing the medical device. [45]

7.5.8 Ophthalmological evaluation

Evaluation of your eyeball, eyelids, eyelashes, and other structures of your eyes by inspection, slit lamp (biomicroscopy), as well as palpation (touch). As part of this evaluation, visual acuity is taken with better corrected visual acuity, the integrity of the ocular surface is assessed, the anterior segment, intraocular pressure is taken, gonioscopy is assessed, and posterior segment (fundoscopy) is assessed.

7.5.8.1 Best-corrected visual acuity

Described in the 7.4.4.4 Best-corrected visual acuity

7.5.8.2 Ocular Surface Integrity

This will be done by means of biomicroscopy using the slit lamp of the research center. An inspection of the cornea, conjunctiva, tear film, and fluorescein eye staining will be done (in section 7.4.4.5 Ocular surface staining).

7.5.8.3 Previous segment

It is the evaluation of the structures of the anterior segment (cornea, conjunctiva, anterior chamber, iris, pupil, lens, aqueous humor). The evaluation will be made by means of the slit lamp.

7.5.8.4 Intraocular pressure

Tonometry is the objective measurement of IOP, based primarily on the force required to flatten the cornea or the degree of corneal indentation produced by a fixed force. Goldmann tonometry is based on the Imbert-Fick principle. [46]

The tonometry will be performed with a Goldmann tonometer, after instillation of the topical anesthetic, with fluorescein and the use of the cobalt blue filter (after the evaluation of the surface staining). 2 samples will be taken and the average will be calculated, which will be recorded in the clinical file.

7.5.8.5 Gonioscopy

It is the assessment of the iridocorneal angle by attaching a gonioscopy lens to the subject's cornea. In some cases the lens will be filled with a high viscosity solution (2% hypromellose) to improve the coupling of it with the subject's cornea.

This evaluation will be carried out only at the baseline visit as part of the comprehensive ophthalmological check-up and will be useful to corroborate the subject's ophthalmological health status.

For the classification of the angle, the Shaffer system will be used.

Board 3. Shaffer's classification

DEGREE	ANGLED APERTURE	DESCRIPTION	RISK OF OCCLUDING
4	45°-35°	Open	Impossible
3	35°-20°	Open	Impossible
2	20°	Narrow	Possible
1	≤ 10°	Extremely narrow	Probable
0	0°	Closed	Occluded

Angular closure or suspected angular closure shall be considered to be Grade 2 or less in more than 180° of the angular circumference.

7.5.8.6 Posterior Segment (Fundoscopy)

Also called ophthalmoscopy. This is the test done with a light and a magnifying glass to look at the fundus (optic nerve and retina) through the pupil. Sometimes the pupil will need to be dilated so that the fundus of the eye can be fully evaluated.

This evaluation will be carried out only at the baseline visit as part of the comprehensive ophthalmological check-up under pharmacological mydriasis and will be useful to corroborate the subject's ophthalmological health status.

7.5.9 Application of Medications During Visits

During the visits to the subjects, different medications or medical devices may be applied to the ocular surface for ophthalmological examination.

Examples:

- Tetracaine 0.5% ophthalmic solution, used to anesthetize the subject's ocular surface and facilitate the measurement of intraocular pressure, application of dyes for stains and placement of the lens for gonioscopy.
- Ophthalmic solution of tropicamide 0.8% and phenylephrine 5%, used to dilate the pupil and better assess the posterior segment.
- Hypromellose ophthalmic solution 2%, may be used by the Investigator for the assessment of gonioscopy.

7.5.10 Delivery of material for the subject

It refers to the delivery of the subject's identification card and the subject's diary. The ID card will serve as an ID with the treatment assignment number, this card may serve as an appointment card. The Subject's Diary serves to keep track of the times the treatment is applied.

7.5.11 Delivery of Study Medication

Once the subject is in the study, the investigational product will be given, and the dosage of the product will be explained (one drop three times a day in the right eye).

7.5.12 Evaluation of concomitant medicinal products

It is the question about the use of medications that they are taking regularly or that they have used in the last month. If you have required injected eye drug therapy, you should ask about the injected drugs in the last 6 months.

7.5.13 Assessment of adherence to treatment

It refers to indirectly evaluating the number of applications during the period between visits. In order to assess the approximate number of drops, the Subject's Journal can be reviewed to find out the applications registered.

7.5.14 Return of Study Medication

It refers to the return made by the research subject of the product under investigation.

7.5.15 Subject Journal Removal

It refers to the delivery made by the research subject of the subject's diary.

7.6 Study Diagram and Timeline

7.6.1 Study diagram

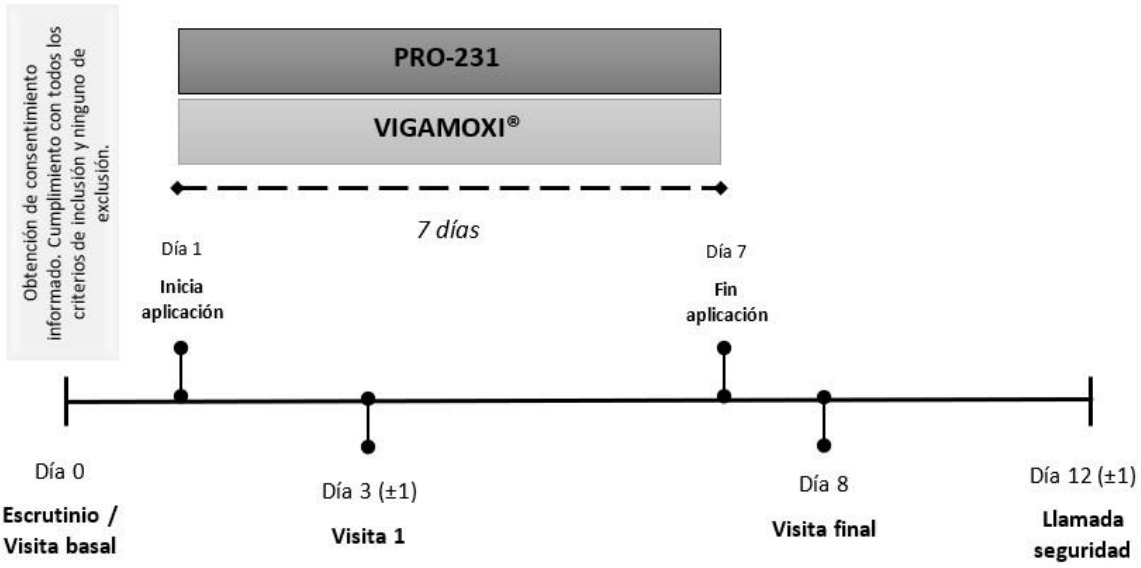


Figure 2. Study diagram

7.6.2 Study timeline

Procedures	Scrutiny / Basal Visit Day 0	Visit 1 Day 3 ± 1	Final Visit Day 8	Call Security Day 12 ± 1
Signing the informed consent form	X			
Medical history	X			
Ophthalmological medical history	X			
Urine pregnancy test	X		X	
Eligibility Criteria	X			
Eye symptoms	X	X	X	
AVMC Measurement	X	X	X	
Previous Segment Evaluation	X	X	X	
Fluorescein staining evaluation	X	X	X	
Intraocular pressure measurement	X	X	X	
Posterior Segment Evaluation	X		X	
Subject and IP code assignment	X			
Delivery of the PIs and indicate the start of application the next day (day 1)	X			
Return of IP			X	
Adherence assessment		X	X	
Evaluation of adverse events	X	X	X	X
Eye Comfort Index	X		X	
Subject Diary Delivery	X			
Return / Evaluation of the subject's Journal			X	
Subject continuity assessment		X		

Abbreviations: AVMC, best corrected visual acuity; PI, research products.

Board 4. Study timeline

7.7 Procedures to be carried out per visit

Listed below are the procedures that will be performed at each visit, which may not show the optimal order for the Research Center. The investigator should order it according to his or her needs, and the needs of the study and sponsor.

In the evaluation of adherence to treatment, it can be reviewed in the subject's diary.

7.7.1 Baseline visit

- Signing of informed consent
- Preparation of general and ophthalmological medical history
- Pregnancy test (where applicable)
- Best-corrected visual acuity measurement
- Previous Segment Evaluation
- Fundus evaluation under pharmacologic mydriasis
- Evaluation of the angle in the anterior chamber (gonioscopy)
- Fluorescein Eye Staining
- Intraocular pressure measurement
- Evaluation of adverse events
- Eye symptoms
- Eye Comfort Index (ILO)
- Subject Code Assignment and Research Product Delivery
- Submission of the subject's diary

7.7.2 Visit 1

- Best-corrected visual acuity measurement
- Previous Segment Evaluation
- Fluorescein Eye Staining
- Intraocular pressure measurement
- Evaluation of adverse events
- Adherence Assessment
- Eye symptoms

7.7.3 Final Visit

- Pregnancy test (where applicable)
- Best-corrected visual acuity measurement
- Previous Segment Evaluation
- Posterior Segment Evaluation
- Fluorescein Eye Staining
- Intraocular pressure measurement
- Evaluation of adverse events
- Eye Comfort Index (ILO)
- Adherence Assessment
- Eye symptoms
- Return of the research product
- Return of subject's diary

7.7.4 Security Call

- Evaluation of adverse events

7.7.5 Unscheduled follow-up visits

At the request of the subject or personnel related to the study, unscheduled follow-up visits may be carried out for the reporting of adverse events or any situation that warrants it. During these visits, all pertinent data on the adverse events reported should be collected and, where appropriate, an appropriate management plan should be established.

7.8 Data collection

7.8.1 Source documents

Source documents are all written or printed records derived from automated processes (e.g., printouts of laboratory results issued by automated analysis equipment) where the information is recorded for the first time and which is part of the permanent records of the subject's history. Examples of source documents are the medical history, clinical evolution notes, laboratory reports, cabinet study reports, nursing notes, follow-up notes, surgery records, etc.

The PI is obliged to accept the monitoring of the information related to the study, audits, review by ethics and research committees, and inspections by the health authority, this obligation implies direct access to the source documents.

7.8.2 Electronic forms of data collection

All data related to the protocol will be captured through an electronic *Case Report Form* (eCRF) by the investigation team staff. The data related to the protocol should NOT be captured directly in the eCRF, but should be transcribed from the corresponding source document. This procedure allows monitoring to verify the information captured in the eCRFs. It is the responsibility of the researcher to ensure that the information is transcribed into the eCRFs in a correct, complete, and timely manner. It is understood that all data captured and sent by the eCRF to data analysis is approved by the Researcher.

7.8.3 Archiving

The data collected in this database is anonymous (it only stores the subject number together with other information of interest). The program used for data capture and storage covers the traceability requirements necessary for the execution of clinical studies. The data collected will be stored by the sponsor or the clinical research organization designated for this purpose and its storage will have a duration of 10 years. The master file will remain in the participating institutions in charge of the PI or its work team and must be safeguarded for at least 5 years.

8. Evaluation and management of adverse events

8.1 Regulation and regulations on adverse events

The registration and reporting of adverse events will be carried out in accordance with the guidelines established in NOM-220-SSA1-2016 and the international guidelines ICH E6. [33][47][48][49]

8.2 Definition of Adverse Event

According to the International Council on Harmonization (ICH), an adverse event (AE) is any unfavorable medical appearance in a clinical investigational subject who is administered a pharmaceutical product, regardless of causal attribution. [47] [48] [49]

Therefore, an AE can be any of the following: any unfavorable and unintentional disease, symptom, or sign (including an abnormal laboratory finding) that is temporally related to the use of a medical product, whether or not it is considered related to such a product; any new illness or exacerbation of an existing disease (worsening of the nature, frequency, or severity of a known condition); relapse of an intermittent medical condition (e.g., headache) not present at baseline; any deterioration in a laboratory value or other clinical test (e.g., electrocardiogram [ECG], x-ray) that is related to symptoms or that results in a change in study treatment or concomitant treatment or discontinuation of study medication. [47] [48] [49]

As defined in the previous paragraph, an adverse event is defined as any event that occurs during treatment with a drug or device. However, the definition can also be applied as any unwanted event that occurs during a clinical trial, including behavioral disorders. [49]

8.3 Use of adverse events as a study safety variable

Measuring the safety of the use of PRO-231 is paramount to the study, therefore, it is considered important to report any unwanted manifestations or diseases that occur during the course of the study, regardless of whether or not the manifestation is considered to be related to the treatment under investigation. [49]

8.4 Definitions relevant to the classification of adverse events

Severity (serious/non-serious), also called seriousness (serious/non-serious). Serious or serious is defined as any event that: results in death, threatens life, requires hospitalization or prolongs hospitalization, is a cause of permanent or significant disability or disability, is the cause of alterations or malformations in the newborn, other medically important conditions (Medically important event

or reaction: That clinical manifestation or adverse event that in the opinion of the doctor may not be immediately life-threatening, result in death or hospitalization, but which could endanger the subject or require medical intervention to prevent the occurrence of any of the criteria listed in the definition of serious adverse reaction). [50]

Severity (mild, moderate, or severe). Mild are those that present with minimal symptoms, do not require treatment or suspension of the medication; moderate, when they interfere with usual activities, without threatening the subject's life, require treatment and may or may not require discontinuation of the medication; severe, those that interfere with usual activities and require pharmacological treatment and discontinuation of the medication. [33][47][48]

Causality. It is the relationship that is assigned between the pharmaceutical product and the adverse event: certainly caused by the pharmaceutical product, there is clear evidence of causality, i.e. the adverse event reappears with the administration of the pharmaceutical product; probably caused by the pharmaceutical product, there is a high suspicion of causality but no direct evidence is available or it is considered unnecessary or dangerous, i.e. the reaction disappears when the pharmaceutical product is discontinued; possibly caused by the pharmaceutical, there is additional information to suggest that the cause may be due to another pharmaceuticals or disease; unlikely to be caused by the pharmaceutical product, there is a clear explanation of the origin due to the underlying disease or the use of another pharmaceutical product; conditional, there is a lack of data to issue a clear causality; non-classifiable, those for which once all possible information has been obtained about the adverse event, it remains unclassifiable. [33] [47] [48] [49]

8.5 Responsibilities of the researcher

It is the responsibility of the Investigator to verify AEs through questioning, pertinent physical examination, assessment of evolution, as well as appropriate medical and pharmacological management; as well as to follow up until the resolution or outcome and definitive discharge of the AE, following the definitions determined in national and international regulations. [33] [47] [48]

In the event of an AE or any event that puts the health and well-being of the subjects at risk, pertinent medical care will be provided, either at the research center or will be referred to the Hospital Center with the highest resolution power with which the research center has a medical care agreement. The PI will notify the sponsor's clinical monitor, according to the times established in national and international regulations. In the case of serious adverse events, it will notify the sponsor and record the corresponding information in the eCRF and in turn, inform the IRB and IC.

The attention of the AEs will be carried out according to the event care diagram (see Figure 3. Adverse Event Care).

The sponsor's final report will include the report of adverse events in compliance with current national and international regulations. [33] [47]

If the research subject debuts during their participation in the study with any chronic adverse event, such as diabetes or systemic arterial hypertension, they will be referred to the competent health professional for chronic treatment. The follow-up and termination of their participation will be in accordance with the stipulations of NOM-012-SSA3-2012. [33]

8.5.1 Recording of adverse events in the electronic case report form

The adverse event registry considers:

- Subject identification information such as: subject number, age, gender, and if applicable specify the eye.
- Information about the causality of the adverse event, its relationship to investigational products, or to another drug related to the study, as appropriate.
- Important date information:
    - Date on which the adverse event occurs.
    - Date on which the Principal Investigator becomes aware of it.
    - Date of resolution or outcome, as applicable.
- Information on diagnosis and clinical management.
- Record the outcome or resolution of the event:
    - Recovered/resolved
    - Recovered/resolved with sequelae
    - Recovering/Resolving
    - Not Recovered/Unresolved
    - Subject who presented death due to the adverse event
    - Subject who presented death and it is judged that the research product may have contributed
    - Subject who presented death and this is not related to the product under investigation
    - Unknown
- Information about the investigational product or product associated with the Adverse Event, Incident, Adverse Incident, AMR or SRAM must be recorded. The information essential for registration is the generic name, distinctive name or code of the investigational product or of the product associated with the undesirable clinical manifestation; it will also be necessary to enter the data concerning the batch number, manufacturing laboratory, expiration date, dose, route of administration, start and end dates of administration and/or consumption, reason for

prescription; according to whether it is an investigational product or drug (protocol in which the subject currently participates) or whether it is a drug that the research subject consumes for the treatment of underlying concomitant diseases or uses for the management of some transitory sign or symptom that does not correspond to the Natural History of the pathology that motivated its entry into the research protocol.

- Indicate whether the removal of the suspected product (of causing the event) eliminates the adverse event. Also indicate if a dose adjustment is made, if the event changes in terms of intensity or seriousness, persistence of the reaction. It is important to indicate whether in those subjects who are exposed again to the product, which had previously been suspended, the AD reappears.
- Information regarding concomitant pharmacotherapy. Indicate the generic name, the dose, the route of administration, start and end dates of its use, as well as the reason for the prescription, regardless of whether it is in accordance with the prescribing information or technical data sheet or is used outside the regulations or what has been authorized by the local, national or international regulatory authority.
- Relevant medical history information. The analysis of the AE considers the information previously narrated, despite the clinical context in which this harmful phenomenon occurs in the participants of the clinical research protocol, is of special interest, so the information about previous conditions, hypersensitivity or allergy phenomena, previous surgical procedures, laboratory analyses or cabinet examinations that have been performed on the participant, etc., that the researcher deems it appropriate to mention may do so.

8.5.2 Adverse Event Tracking

The Principal Investigator will provide care and follow-up of the adverse event presented by the participant until its outcome, according to what is referred to in the following section.

8.5.2.1 Diagnosis against signs or symptoms

Whenever possible, an AE should be assessed/reported as a diagnosis and not as a sign or symptom (e.g., liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). If a diagnosis cannot be made from the signs or symptoms, then each sign or symptom should be recorded as an AE. If a diagnosis is made later, all AEs reported as signs or symptoms should be nullified and replaced by a single adverse event based on the diagnosis, with an onset date that corresponds to the onset date of the first symptom or sign of the eventual diagnosis.

8.5.2.2 Adverse events secondary to other adverse events

In general, AEs secondary to other AEs (cascading events or sequelae) should be identified according to the primary cause, with the exception of serious events. A medically important AE secondary to an AE that is separated in time from the initial event should be reported as a separate event in the EA section of the eCRF. For example:

If vomiting results in mild dehydration without additional treatment in a normal adult, vomiting should only be reported on the eCRF.

If vomiting results in severe dehydration, both events should be reported separately on the eCRF.

If severe gastrointestinal bleeding leads to renal failure, both events should be reported separately on the eCRF.

If a dizziness event leads to a fall and subsequent fracture, then all three events should be reported separately in the eCRF.

If neutropenia is accompanied by infection, both events should be captured independently in the eCRF.

All EAs must register separately if there are concerns regarding the association of events.

8.5.2.3 Persistent or recurrent adverse events

A persistent adverse event is one that spreads continuously without resolution between different points of assessment of the subject. Such events only need to be registered once in the eCRF. The initial severity (intensity or degree) of the event will be recorded at the time of the first AD record. If a persistent AE becomes acute, the maximum severity should be recorded in the appropriate section of the eCRF. If the event becomes a serious event, it must be reported no later than 24 hours after the knowledge of the change in the status of the event. The eCRF section should be updated to reflect the serious status, and will record the date on which the event became serious, thus completing all relevant serious EA reporting data.

A recurrent AE is one that resolves between assessment points and subsequently reappears. Each recurrence of the EA must be recorded separately in the eCRF.

8.5.2.4 Abnormal vital signs

Not all abnormal vital signs qualify as AS. For an abnormal vital sign to be reported as an AD, it must meet any of the following criteria:

- Be accompanied by clinical symptoms. It results in a change in treatment (dose modification, treatment interruption, etc.).
- The report of abnormal values should focus on obtaining a diagnosis and not just a description of the abnormality.

8.5.2.5 Death

Death should be considered an outcome and not an event. The event or condition that caused or contributed to the fatal outcome should be recorded as the EA in the eCRF section.

8.5.2.6 Pre-existing medical conditions

A pre-existing medical condition should be recorded as AE only if the frequency, severity, or characteristics of the condition worsen during the study.

8.5.2.7 Hospitalization or prolonged hospitalization

Any AE that results in hospitalization or prolongation of hospitalization must be documented or reported as a serious AE with the following exceptions:

Hospitalization for pre-existing conditions as long as the following are met:

Hospitalization was planned before the study.

8.5.2.8 Pregnancies, miscarriages, and birth defects

Fertile women should contact their doctor to report any suspected pregnancy during the study. A pregnancy report must be issued and the sponsor must be informed immediately. Monitoring of the subject should continue until the outcome of pregnancy. Pregnancy is not by itself an AE.

Any abortion should be classified as a serious AE.

Any birth defect or birth defect in a product from a woman who received the study drug should be classified as a serious AE.

8.5.3 Procedures for a serious adverse event

The process of attention to the adverse event considers the following stages:

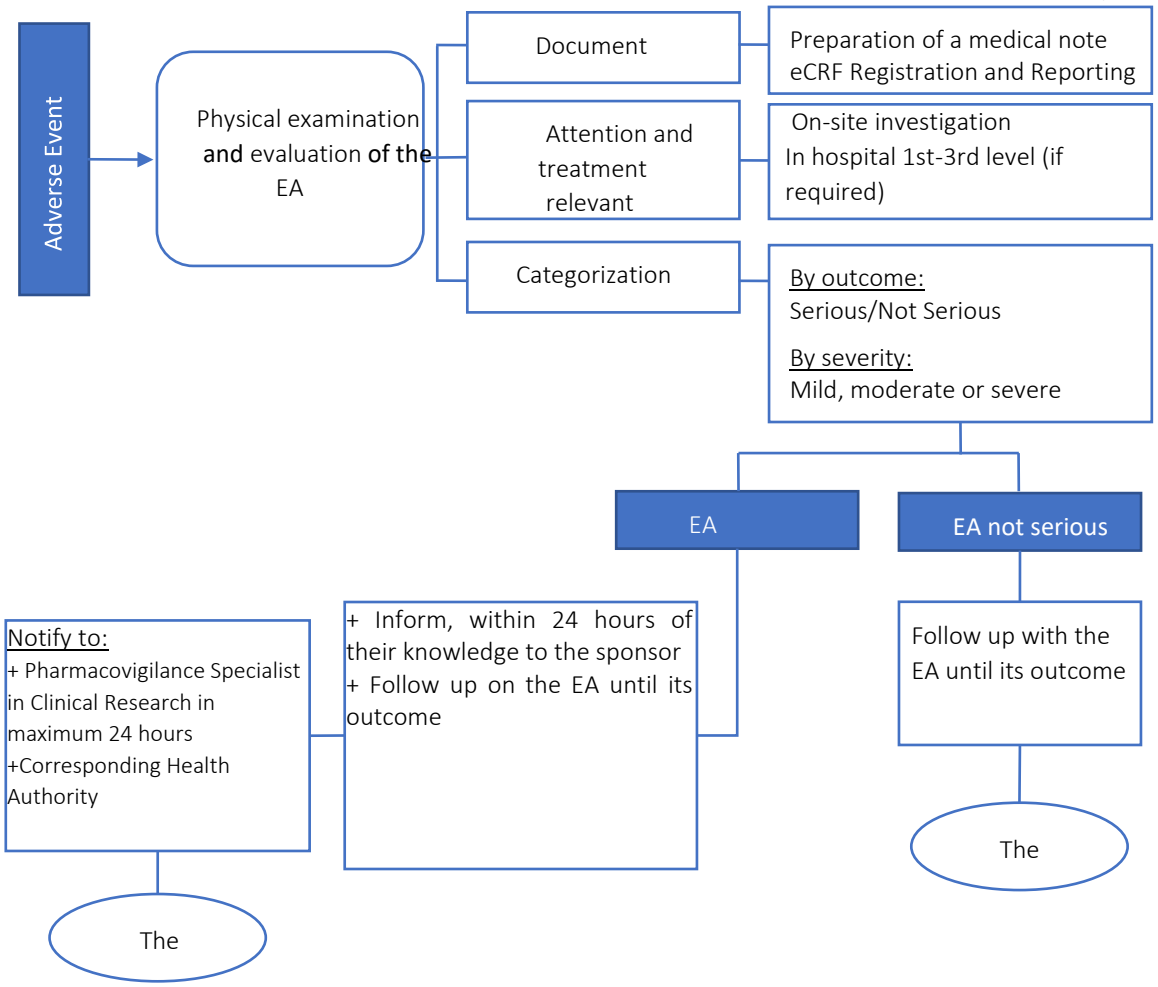


Figure 3. Adverse Event Care

During the development and conduct of this study, undesirable harmful events or adverse reactions/incidents, of medical implication, may occur in the research subject, which do not necessarily have a causal relationship with the investigational products. These harmful phenomena can occur during the use of investigational pharmaceutical products at doses authorized for use in humans by a local, national or international regulatory authority. However, it may be suspected that the investigational product causes some unwanted clinical manifestation. AEs, Incidents, Adverse Incidents, ADRs or SRAMs to one or more pharmaceutical products can occur during the systematic evaluation of the participants (on the days when the clinical review is scheduled, according to the schedule of activities) or suddenly, in such a way that:

1. The investigator must be the first person to whom the subject notifies that he or she has developed or presented any harmful phenomenon of a clinical nature during his or her participation in this study.

2. According to his clinical judgment; Based on the pertinent physical examination, interrogation, etc., as well as the analysis of the information available in the medical literature and what is referred to in the Investigator's Manual, Information to prescribe

or Summary of the Comparator Drug, the principal investigator determines the pertinent care of the harmful event/reaction.

3. Such care can be in the research center or in the hospital with the greatest resolution power. In such a way that, in the event that the subject is sent by the PI to a hospital, he or she attends through a referral system. The reference can be with a card that identifies the subject as a study participant and links him or her to the pre-established agreement with the institution, or through a medical reference note issued by the Principal Investigator. Laboratorios Sophia, S.A. de C.V., will pay the expenses for the medical care of the participating subject, when the adverse event is associated with or related to the product under investigation.

4. Taking the clinical information collected, either during the care provided at the research center or that provided by the treating physician(s) in the hospital, the PI will record the AE in its clinical note, stating the seriousness, intensity (mild, moderate or severe) and relationship with the investigational product.

5. The PI must migrate the relevant data to the eCRF and its respective adverse event section. By virtue of the fact that, in cases of serious adverse events, the clinical monitor of the study must be notified within 24 hours after learning of it, so that in turn it informs the Clinical Team and the Pharmacovigilance and Technovigilance Unit of Sophia Laboratories (UFTLS), and that the CEI/CI is subsequently notified. Non-serious adverse events will be recorded and treated appropriately and the safety profile of the investigational drug or drug will be reported to the appropriate regulatory authority in the final report of the clinical trial.

The recording of the outcome of the AE depends substantially on the follow-up that the Principal Investigator performs on the subject, since it is expected that most of the harmful phenomena (see subsection 2.5 Risk-benefit assessment and consult the Investigator's Manual) are ophthalmic in nature, however, there may be systemic alterations. Therefore, in the opinion of the researcher, the withdrawal of the participant or their permanence will be considered.

8.5.4 Causation assessment

Causality assessment is the methodology used to estimate the probability of attributing the observed adverse event to a pharmaceutical product. It considers probabilistic categories according to the available evidence and the quality of the information, based on the national pharmacovigilance and technovigilance regulations, the World Health Organization and the Uppsala Monitoring Center. [33][51]

An adverse event may or may not be related to the clinical trial. A causal relationship means that the intervention caused (or is reasonably likely to have caused) the adverse event. This usually implies a relationship between the time of the intervention and the adverse event (e.g., the adverse event occurred shortly after the subject under investigation received the intervention). [49]

For all adverse events, the Principal Investigator is responsible for examining and evaluating the subject to determine the association of the event with the clinical study and the intervention, whether related to the experimental treatment, concomitant, surgical procedure, or diagnostic procedures performed during the study. [49]

Accepting that the adverse event is related to the clinical study requires a plausible mechanism of action, that is, that there is a logical sequence between the event that occurred and the intervention that caused it. In some cases, it is helpful to know the opinion of other doctors directly or indirectly involved in the research; as well as whether the subject considers that a relationship exists or not. [49]

UFTLS may employ the causation categories described by *the Uppsala Monitoring Centre*, to categorize the likelihood of adverse event to the investigational product or concomitant treatments or treatments used during visits: [33] [51]

- Definitive (certain): a clinical event, including alterations in laboratory tests, that manifests itself with a plausible temporal sequence in relation to the administration of the drug, and that cannot be explained by concurrent disease, or by other drugs or substances. The response to drug withdrawal (withdrawal) must be clinically plausible. The event must be definitive from a pharmacological or phenomenological point of view, using, if necessary, a conclusive re-exposure (challenge) procedure. [33] [51]

- Probable: A clinical event, including alterations in laboratory tests, that manifests itself with a reasonable time sequence in relation to the administration of the drug, that is unlikely to be attributed to concurrent disease, or to other drugs or substances, and that a clinically reasonable response is presented upon withdrawal. It is not necessary to have re-exposure (challenge) information to assign this definition [33] [51]

- Possible: A clinical event, including alterations in laboratory tests, that manifests itself with a reasonable time sequence in relation to the administration of the medicinal product, but which can also be explained by concurrent disease, or by other drugs or substances. Information regarding the withdrawal of the drug may be missing or unclear. [33] [51]

- Unlikely: A clinical event, including alterations in laboratory tests, that manifests itself with an unlikely temporal sequence in relation to the administration of the drug, and that can be more plausibly explained by concurrent disease, or by other drugs or substances. [33] [51]

- Conditional/Unclassified: A clinical event, including alterations in laboratory tests, reported as an adverse reaction, for which further data are essential for appropriate evaluation, or additional data are under review. [33] [51]

- Non-assessable/Unclassifiable: A notification that suggests an adverse reaction, but cannot be judged because the information is insufficient or contradictory, and that cannot be verified or completed in its data. [33] [51]

Thus, the degree of certainty to establish the investigational product as the causal agent of the harmful phenomenon that occurs to the subject of the clinical study, can be indicated directly by the Principal Investigator based on his clinical experience or by applying the categories of causality described by *the Uppsala Monitoring Centre*. It is important that the researcher and the UFTLS take into account the following arguments in favor of the causal relationship:

a) Strength of association, which refers to the number of cases in relation to those exposed.

b) The consistency of the data, i.e. the presence of a common characteristic or pattern.

c) The exposure-effect pattern, which determines the relationship with the site of appearance, time, dose and reversibility after deletion.

d) Biological plausibility, which refers to the possible pharmacological or pathophysiological mechanisms involved in the development or presentation of the adverse event.

e) Experimental findings, e.g., the appearance of abnormal metabolites or high levels of the drug or its biotransformation product.

f) Analogy, which refers to the experience gained with other related drugs, adverse reactions frequently produced by the same family of pharmacological agents.

g) Nature and characteristics of the data, i.e. objectivity, accuracy and validity of the relevant documentation. [52]

9. Study Monitoring

The study sponsor is responsible for monitoring the study. Monitoring activities include, but are not limited to: general safety monitoring, general study quality monitoring, monitoring by study site, detection monitoring, reporting and tracking of adverse events, monitoring for resolution of discrepancies in data capture, etc.

Responsibility for monitoring activities and ultimate responsibility for monitoring rests with the sponsor.

The details of the monitoring activities are specified in a separate document from this protocol in a Monitoring Plan.

9.1 Monitoring the Study Site

The research center participating in the study will be monitored. For each centre, at least one start visit and one closing visit must be carried out, which does not exclude the carrying out of one or more follow-up visits between these two mandatory visits.

The initial visit must be carried out before the inclusion of the first participant in that center; In it, the monitor will verify that the material to be used during the study has been received and that the personnel who will participate in the study activities have been trained on the study, as well as verify that the regulatory requirements and applicable standard operating procedures are met.

At the follow-up visit(s), the monitor will conduct a review of the study documents to confirm that the applicable research protocol and standard operating procedures are being followed, data completion is complete and timely, and that adverse event reports are being conducted appropriately. At each visit, the monitor will discuss the findings with the researcher and define the actions to be taken.

The closure visit will take place at the end of the study, once the last site participant has been discharged from follow-up. On this visit the monitor will verify that the site has all the necessary documents for archiving, that all biological samples have been analyzed, that all IP (used and unused) has been returned to the sponsor, and that all unused material has been recovered.

Details on monitoring are set out in the corresponding plan.

9.2 Audit and quality assurance

To ensure compliance with Good Clinical Practice (GCP) and all applicable regulatory requirements, Laboratorios Sophia, S.A. de C.V. may conduct quality assurance audits. Regulatory agencies could also conduct a regulatory inspection of this study.

Details about the audit process are set out separately in the Audit Plan.

They may take place at any time before, during, or after the conclusion of the study. If any audit or inspection is conducted, the investigator and the institution shall agree to allow the auditor/inspector direct access to all relevant documents, and shall allocate their time and that of their staff to the auditor/inspector to discuss the findings and any pertinent problems. In the event that the audit has not been scheduled by the sponsor, the facility must notify Laboratorios Sophia, S.A. de C.V. immediately.

10. Sample size calculation and statistical analysis

10.1 Sample Size Calculation

Bacterial conjunctivitis is commonly self-limiting, resolving after about 10 days, and can rarely last more than 3 weeks. Its most common treatment is the use of antibiotics with a broad spectrum of action on the variety of ocular pathogens. Proper management of bacterial conjunctivitis reduces the time to resolution of the disease, minimizes the risk of sequelae, helps prevent the spread of infection, and decreases the time the patient can spend away from work or school. Moxifloxacin hydrochloride is a fourth-generation fluoroquinolone, its use in topical ophthalmic presentation for the treatment of bacterial conjunctivitis has been approved since 2003 in the United States. Moxifloxacin is one of the most potent fluoroquinolones evaluated for ophthalmic use for bacterial eradication. There is sufficient evidence on the safety and tolerability of moxifloxacin. Commonly reported AEs are eye pain, eye burning/irritation, hyperemia, and tearing; most of them of mild intensity and transient.[53] [54, 55] [54] [53, 55, 56, 57, 58]

Considering this information and the primary objective of the study; To evaluate the safety and tolerability of PRO-231 ophthalmic solution (moxifloxacin 0.5%) on the ocular surface of clinically healthy subjects, the following proposal was made for the sample size.

10.1.1 Calculation methodology

Although sample estimation in phase 1 studies is common in treatments whose toxicity/risk is high, as it is in clinical studies for the management/survival of cancer; It was considered pertinent to estimate the sample size according to the safety profile reported in the literature after the use of moxifloxacin hydrochloride. Considering the primary safety variables of the protocol: [59]

- Incidence of AD
- Changes in ophthalmologic signs
- Changes in the AVMC
- Changes in fluorescein staining of the ocular surface

The equation was used to compare two proportions: two samples, two sides of equality. This calculation is useful when you want to test whether the proportions of two groups are different (Incidence of AD in VIGAMOXI® is different from the incidence of AD in PRO-231). The following formulas are used for the calculation to compute the sample size and power respectively: [60]

$$n_A = kn_B \; y \; n_B = \left(\frac{p_A(1-p_A)}{k}\right)\left(\frac{z_{1-\alpha/2} + z_{1-\beta}}{p_A - p_B}\right)^2$$

$$1-\beta=\Phi\left(z-z_{1-\alpha/2}\right)+\Phi\left(-z-z_{1-\alpha/2}\right), z=\frac{p_A-p_B}{\sqrt{\frac{p_A(1-p_A)}{n_A}+\frac{p_B(1-p_B)}{n_B}}}$$

Where:

k = nA/nB: ratio between the sample sizes of the two groups

Φ: Function of Standard Normal Distribution

Φ -1: The standard normal quantile function

α: error tipo I

β: error tipo II

The proportion of subjects who had at least one AE (ocular and non-ocular) was considered in the study by Garg et al. (2015), where they evaluated the clinical and antibacterial efficacy, as well as the safety of besifloxacin ophthalmic suspension compared to moxifloxacin 0.5% (besifloxacin; n=61, moxifloxacin; n=62). A total of 6 ocular AEs were present in subjects treated with moxifloxacin and at least one AE occurred in 3 patients. The most common AE was conjunctivitis (3.2%). For the expected proportion of subjects of PRO-231, 50% was used, which is a conservative value and gives larger samples. The calculation was made using the free software R (R Core Team, 2021), using a code available online for this purpose. [56] [61] [62]

10.1.2 Size Calculation

PRO-231 ophthalmic solution is expected to be safe in its ophthalmic application as it presents an incidence of AE similar to that presented by VIGAMOXI.®

On this basis, a sample of 17 ophthalmologically and clinically healthy subjects was estimated. This value increased by 10% considering possible losses. Based on this consideration, 19 subjects per arm (38 subjects in total) are required to provide one eye (OD) to respond to the primary objective of the study.

10.2 Clinical Data Management

The *clinical data management* (or clinical data management, CDM), allows the generation of high-quality, reliable data with statistical value. CDM is the process of collecting, cleaning, and managing the information of the subjects of a study in accordance with regulatory standards (guidelines 21 CRF Part 11, ICH and GCP). It covers the design of the electronic case report form (eCRF), comments on the eCRF, database design, capture (*data entry*), validation (*source document verification*, SDV), discrepancy management (*You want*), medical coding (*medical coding*), extraction (*soft lock*) and database shutdown (*hard lock*) .[63]

In accordance with roles and responsibilities, multiple users can be created, whose types of access to the eCRF can be limited to capture (principal investigator, IP), medical coding, database design, or quality control (*quality check*) . The handling of discrepancies will be carried out based on the flow in the [63, 64]Figure 4.

The following roles will be included in the CDM team:

- Data Manager
- Database Designer/Programmer
- Medical *Coder*
- Clinical Data Coordinator
- Quality Control
- Capturist (*data entry associate*)

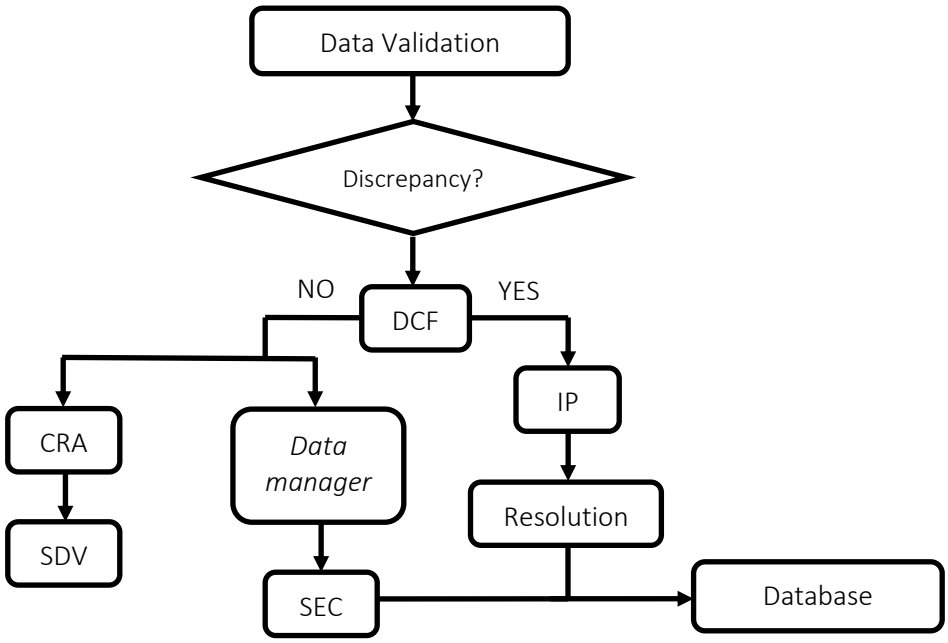


Figure 4. Handling discrepancies.

(DCF, medical note; CRA, clinical monitor; PI, principal investigator; SEC, self-evident correction) [63]

10.3 Statistical methodology

The statistical analysis plan (PAE) was developed considering the evaluation criteria described in the study protocol.

The statistical analysis will be carried out by personnel of Laboratorios Sophia, S.A. de C.V. Specialized statistical software (SPSS version available [IBM Corporation, Armonk, NY, USA] or R [*The R Foundation for Statistical Computing*; http://www.R-project.org]), for Windows. The coding will be done using consecutive numbers. The data will be collected and sorted in an Excel spreadsheet (Microsoft® Office). The data will then be exported to the platform of the selected statistical package. The variables will be categorized according to their nature (see Board 2). The statistical tests used will be following the corresponding assumptions for parametric statistics as long as the assumption of normality is met (Shapiro-Wilk, p>0.05).

10.3.1 Population Analysis

The statistical analysis will be presented to give a general summary of the subjects entered into the study and a view of the safety and tolerability of its results. The data provided by the research site will be summarized for this purpose, according to its nature. The Shapiro-Wilk test will be performed to find out if the quantitative data are distributed in a normal way (p>0.05).

The results of the quantitative variables will be presented in measures of central tendency: mean, standard deviation and/or ranges. Changes in the AVMC and ICO will be expressed as continuous variables. Whereas, the incidence (number) of AEs will be expressed as a discrete variable.

The result of the nominal and ordinal qualitative variables will be presented in frequencies, proportions and/or percentages. For these, tables of frequencies p x q will be constructed. All percentages will be presented with a decimal.

The level of difference to consider significance will be an alpha (α) of 0.05 or less, the 95% CI will be calculated for these differences. The triangulation between the type of variable and the measurements is shown in Table 5. [63]

The operational definition of the study for hypothesis testing will be as follows:

Hypothesis testing	value	Result	Interpretation
P value	≤ 0.05	H_0 rejected	Both treatments are different in terms of safety and tolerability
95% CI	Does not include zero		
P value	≥ 0.05	H_0 accepted	Both treatments are similar in terms of safety and tolerability (they are not different)
95% CI	Includes zero		

Both conditions for hypothesis testing (p-value and 95% CI) must be met, otherwise the result will be interpreted as **"absence of evidence",** not as **"evidence of absence".**

Board 5. Triangulation of concepts.

Variable Type	Variable	A1	A2	B1	C1	C2	C3	C4	D1	E1	F1	F2	F3	F4
Selection														
A1	Demographic	D												
A2	Medical History/Selection Criteria		D											
Basal														
B1	Comprehensive ophthalmological evaluation		T	D	TB	TB	B	T		TB				
Primary Security														
C1	Incidence of unexpected-related AEs				TB					TB				
C2	Incidence of conjunctival hyperemia and chemosis			T		TB				TB				
C3	AVMC			B	TB		B			TB				
C4	Corneal and conjunctival stains with fluorescein			B	TB			B		TB				
Primary Tolerability														
D1	ICO								B					
Secondary Security														
E1	Incidence of AE*			T				TB						
Secondary Tolerability														
F1	Presence of burning									B	TB			
F2	ETS Presence									B		TB		
F3	Presence of pruritus									B			TB	
F4	Presence of tearing									B				TB

Abbreviations: AVMC, best corrected visual acuity; B, bivariate analysis; D, descriptive statistics; AE, adverse event; ICO, eye comfort index; SCE, foreign body sensation; T, contingency table p x q. *EAs related or not, with the research product.

10.3.2 Safety and tolerability analysis

10.3.2.1 Analysis for Primary Variables

The analysis of the primary outcome variables of safety and tolerability will be carried out in the per-protocol population (PP), defined as any subject with an adherence ≥ 80% determined by the subject's diary and corroborated by the final weight of the research product to which it was assigned (PRO-231 or VIGAMOXI)® with respect to its initial weight. and that they conclude their participation without deviations from the study protocol.

The Shapiro-Wilk test (S-W) will be used to determine if the distribution is normal in the data obtained in each study group (p>0.05). If the normality in the distribution of the data is verified, to rule out differences between groups, the Student's t-statistic will be used for independent groups, while intragroup differences will be analyzed with the Student's t-test for repeated measures.

In case of p<0.05 in S-W, the statistical analysis to rule out differences between groups will be performed by means of the Mann-Whitney U test for quantitative variables, while intragroup differences will be performed by Wilcoxon's rank test.

For qualitative variables, Pearson's X2 (Chi-square) or Fisher's exact test will be used in expected values less than 5. Dichotomous variables will be analyzed by the McNemar test when applicable.

10.3.2.2 Analysis for secondary variables

The analysis of the secondary outcome variables of safety and tolerability will be carried out in the safety population, defined as any subject who has received at least one dose of the investigational product (PRO-231 or VIGAMOXI)® regardless of their adherence to it or adherence to the protocol (intention-to-treat population, ITT).

For the analysis of these variables, the same primary analysis will be carried out as long as they have the necessary measurements to do so.

10.4 Other analyses

The final report of the results will be shown in tables or graphs, as appropriate.

10.4.1 Procedure for handling missing data

There is no imputation procedure for missing data.

11. Ethical considerations

11.1 Approval of the committees

The present study will be conducted in accordance with the principles of the Declaration of Helsinki, World Medical Association 2013. Nuremberg Code; Nuremberg Trial by the International Tribunal of Nuremberg, 1947. Belmont Report, National Commission for the Protection of Biomedical and Behavioral Research Subjects, 1979. It will be conducted in accordance with the scientific and technical requirements necessary for the registration of medicines for human use by the International Council for Harmonisation (ICH) Guide to Good Clinical Practice. Council for International Organizations of Medical Sciences (CIOMS, 2002) International Ethical Guidelines for Biomedical Research in Human Subjects. Council for International Organizations of Medical Sciences (CIOMS, 2008) International Ethical Guidelines for Epidemiological Studies. The Research Ethics Committee and the Research Committee will evaluate the protocol before conducting the study and will issue their approval or possible modifications for its performance, these Committees must be notified of any significant changes to the protocol. In addition to the above, the current regulations of the regulatory authority will also be complied with.

The personnel authorized by the sponsor will submit to evaluation by the Research Ethics Committees, Research Committees, and when applicable to the Biosafety Committee the essential documentation of the research project: research protocol, informed consent form, researcher's manual, subject material, as well as other documents additionally requested, according to local requirements, applicable by the regulatory authorities.

The study will not begin at the research center if there are no confidentiality agreements and economic proposal from each of the principal investigators, duly signed and without having previously obtained the favorable opinion and/or approval of the Research Ethics Committees, Research Committees, and when applicable by the Biosafety Committee. Corresponding.

The study will not begin without having complied with the relevant local, national or international regulatory requirements and without having the corresponding health authorization.

The study is considered to be a study with a greater than minimum risk, in accordance with the Regulations of the General Health Law on Health Research, Title Two, Chapter I, Article 17, Section III, published in the Official Gazette of January 6, 1987.

11.2 Amendments to the protocol

The amendment procedure will be pertinent when there is a need to make any change to a document that is part of the research project or protocol, derived from variations in the methodological structure, substitution of the principal investigator or in the face of the identification of risks in the research subjects. The documents that may be amended will be: protocol, form of informed consent, researcher's manual, documents for the subject, measurement scales and schedule of activities.

Any amendment must be approved by the sponsor and/or the principal investigator, the amended document(s), once reviewed and approved by the CEI and the IC or, when applicable, by the Biosafety Committee, (entities that issued the initial favorable opinion for the conduct of the research) will be sent for authorization by COFEPRIS.

Amendments that substantially modify the protocol confer an additional or different risk on the research subjects must be approved by the aforementioned Committees. It is the investigator's responsibility to take action in situations that require immediate action to avoid unnecessary harm to study participants.

The principal investigator is responsible for communicating to the Research Ethics Committee any amendments to the protocol that may eventually affect the rights, safety, or welfare of research participants. Likewise, it must inform of any situation or new knowledge that will show a greater risk for the participants, the premature termination or suspension of the study, the reasons and the results obtained so far. It must also report on the conclusion of the study, upon completion of the research protocol.

11.3 Early Study Termination

The study may be temporarily suspended or terminated prematurely if there is a sufficiently reasonable cause. Written notice, documenting the reason for the suspension or early termination, must be delivered by the party executing the suspension. The PI should promptly inform the study participants, the IC, and the IRC providing the reasons.

Situations in which study suspension or early termination will be contemplated include, but are not limited to:

1. The presence of serious adverse events in more than 10% of participants in a study group.
2. The regulatory authority (COFEPRIS) considers it for security alerts.
3. The Sponsor determines it for its convenience or eventualities such as: financial support, manufacturing errors, etc.
4. The identification of unexpected risks to the participants, which are significant or unacceptable.
5. Obtaining new relevant safety information.
6. Insufficient adherence to the requirements of the protocol.
7. The data obtained are not assessable or are not sufficiently complete.
8. The determination that the primary objective has been achieved.

9. The determination of futility.

In the event of suspension, the study may be resumed once the situations that led to the suspension have been corrected; as long as this justification is sufficient for the sponsor, CI, CEI and regulatory authorities.

11.4 Informed Consent

The FCI contains complete and understandable information about the study and the investigational product, in accordance with the applicable regulations in force and Good Clinical Practice.

The FCI will be considered as a source document and will be filed as such. The site's principal investigator is responsible for ensuring that all new versions of the informed consent are submitted to the appropriate approvals (the same as those to which the original informed consent form was submitted) and that the most current approved version is the one presented to the study subjects.

11.4.1 Obtaining

Informed consent must be obtained before the subject undergoes any procedure indicated in the protocol. For this purpose, the informed consent form must be signed.

Written consent documents will incorporate the elements of informed consent described in the Declaration of Helsinki and the ICH Guide to Good Clinical Practice and will be in compliance with all applicable laws and regulations.

The PI, or the study staff delegated by him/her, will provide the prospective participant with all the information regarding the characteristics of the study, its potential benefits, risks, objectives, and procedures.

This information will be in a language understandable to the subject, it will be explained to the subject that he or she has the right to interrupt his or her participation in the study at any stage, without this affecting the relationship with the researcher and/or his or her future assistance. Informed consent will be put to the consideration of the potential participant; He must have enough time to analyze each and every one of the aspects mentioned above and in case he has any doubts, it will be clarified by the person in charge of obtaining the informed consent.

Once the participant agrees to participate in the study, he/she must sign and date the informed consent form in the presence of two witnesses who are or are not related to the study subject, who will participate during the informed consent process and will sign guaranteeing that the process was carried out prior to any study procedure. that the information of the study was clearly explained and doubts were clarified if any.

In the event that a subject is illiterate, acceptance will be with his or her fingerprint, and in the event that the subject is unable to give adequate written informed consent, a representative of the "legally

authorized" subject may provide such consent for the subject in compliance with applicable laws and regulations.

In the same way, the PI, or the study staff delegated by him, must sign and date this consent.

The FCI must be signed in duplicate by all those involved, one copy will be filed in the researcher's folder and the other will be given to the participant. The PI or delegated personnel must document the process of obtaining the Informed Consent by means of a detailed, precise and contemporary medical note, specifying the signed version, the date on which the document was signed and how the process was carried out.

11.4.2 Special considerations

The procedures that will be performed during the conduct of the study do not pose an additional risk that should be considered apart from the procedures listed for informed consent.

11.4.3 Modifications to informed consent

Any changes to the FCI constitute an amendment to this document and must be submitted for approval to the Research, Research Ethics and COFEPRIS Committees.

Such amendments may be implemented only after obtaining the written approval of the Research Ethics Committee and authorization from COFEPRIS (as applicable), except for an amendment that is required to eliminate an immediate danger to study subjects.

A process of re-consent of each subject affected by the amendment must be carried out under the same conditions as those described above, in order to communicate the new information contained in the document to them in a timely manner. The subject will be given a signed original of the amendment and the researcher will keep the second original.

11.5 Confidentiality

All documents and information provided to the research center by the sponsor are strictly confidential. PI expressly agrees that data about your professional and clinical experience, provided to the sponsor on paper and stored in electronic form, is solely for use related to your activities with the clinical trial sponsor, in accordance with Good Clinical Practice.

The PI agrees that he and his team members will use the information only within the framework of this study, to carry out the protocol. This agreement is binding as long as the confidential information has not been disclosed to the public by the sponsor.

The clinical study protocol provided to the PI may be used by the PI and his team to obtain the informed consent of the subjects for the study. The clinical trial protocol, as well as any information taken from it, should not be disclosed to other parties without the written permission of the sponsor.

The PI will not disclose any information without the prior written consent of Laboratorios Sophia, S.A. de C.V., except to the representatives of the Competent Authorities, and only at their request. In the

latter case, the investigator undertakes to inform Laboratorios Sophia, S.A. de C.V. before disclosing the information to these authorities.

The PI will fill out and maintain a record of the selection of the subjects, as well as the identification and enrollment list of each of the subjects participating in the study. The investigator agrees to give on-site access to the auditor and/or representatives of the Competent Authorities. The information will be processed in compliance with professional secrecy.

In the eCRF and all communications related to study subjects, they will identify them only by the study subject's identification number, either by the scrutiny number or the assignment number. The information collected in this study will be exchanged between the sponsor and the research center, and must be treated confidentially. The Health Authority, the CEI, the IC, the sponsor, the monitors/auditors and third-party auditors will be the only bodies authorized to review the study documentation. If publications arise from this research project, in no case will they contain information on the identification of the study subjects. If the results of the study are published, no personal information of the study subjects will be revealed.

The protection of personal data will be carried out in accordance with the corresponding regulations in force.

11.6 Conflict of interest

The independence of the conduct of the study and its results from any current or perceived external influences is critical. For this reason, any current conflict of interest of any person who has a role in the design, conduct, analysis, publication or any aspect of this study will be declared. Furthermore, those who have a perceived conflict of interest will be asked to handle it in a manner appropriate to their participation in the study.

11.6.1 Declaration of Interests

The PI undertakes to make a declaration of financial interests as well as conflict of interest prior to the start of the study.

11.7 Access to Information

The final database of the study will be the property of Laboratorios Sophia, S.A. de C.V. and its access will be restricted. The PI will not have access to it, unless it has prior written authorization from the sponsor.

The information obtained, which is relevant to the safety of the subjects participating in the study, must be immediately shared with the research center, so that it can be notified to the study subjects.

11.8 Ancillary and post-study care

Upon completion of the study and closure of adverse events in accordance with section: 8, the sponsor shall not extend care to the research subject.

12. Biosecurity aspects

NO BIOSECURITY IMPLICATIONS

This protocol, entitled: "Phase I clinical study, to evaluate the safety and tolerability of the ophthalmic solution PRO-231, versus VIGAMOXI,® on the ocular surface of ophthalmologically and clinically healthy subjects", and number: SOPH231-1221/I has no biosafety implications, since infectious-contagious biological material; pathogenic strains of bacteria or parasites; viruses of any type; radioactive material of any type; animals and/or cells will NOT be used and/or genetically modified plants; toxic, dangerous or explosive substances; any other material that endangers the health or physical integrity of the research centre's staff or research subjects or affects the environment. It is also declared that this project will not carry out cell, tissue or organ transplant procedures, or cell therapy, nor will laboratory, farm or wildlife animals be used.

13. Posting Policy

13.1 Final Report

Once the statistical analysis is completed, the final report will be written with the results obtained, by the Team of the Regional Management Department of Medical Affairs of Laboratorios Sophia, S.A. de C.V. This report will be prepared following the recommendations of the E3 *Step 4 Guide* of the ICH.

13.2 Communication of results

Regardless of the results of the study, Laboratorios Sophia, S.A. de C.V., is committed to communicating the final report of the study to the principal investigators and COFEPRIS. These results will also be shared with the IC and the IEC. It will be the responsibility of the PI to communicate it to the research subjects.

Laboratorios Sophia, S.A. de C.V. will maintain at all times the rights over the publication and disclosure of the information contained.

13.3 Publication of results

Laboratorios Sophia, S.A. de C.V., acting as the sponsor of the study, assumes full responsibility for its function and retains exclusive ownership rights over the results of the study, which it may use in any way it deems appropriate.

The PI undertakes not to publish or communicate data collected from the study, unless there is the prior written agreement of Laboratorios Sophia, S.A. de C.V. Any manuscript derived from the data obtained with this protocol must be submitted to review by the sponsor before any attempt to submit it for publication in any scientific journal or congress.

However, in the event that the sponsor is in the process of filing a patent application on the results of the study, the sponsor may delay its publication or communication of the results of the study until the date of registration or whenever it deems appropriate.

The assignment of authorship of publications, which is the responsibility of the sponsor, will be the prerogative of the latter. However, the express authorization of the people who are invited to participate as authors must be obtained. Authors have the right to review the manuscript prior to its publication, as well as to issue comments and suggestions in this regard, such comments must be delivered within the first 15 calendar days from the date on which the project is received.

14. Financing and Insurance

14.1 Compensation to Study Participants

Subjects who participate in the study will not receive financial compensation for their participation in the study. However, the subjects will receive financial support for travel expenses in each scheduled visit to which they attend punctually. Such support, as well as the amount, may be specified in the form of informed consent.

14.2 Study Insurance

In accordance with current local regulations, Laboratorios Sophia S.A. de C.V. has contracted a civil liability policy, in order to comply with the responsibility of providing medical treatment and compensation to which a subject would be legally entitled, in the case of damages directly caused by this research.

In the event of a medical emergency, the research center must have personnel, material, equipment and procedures for its immediate management.

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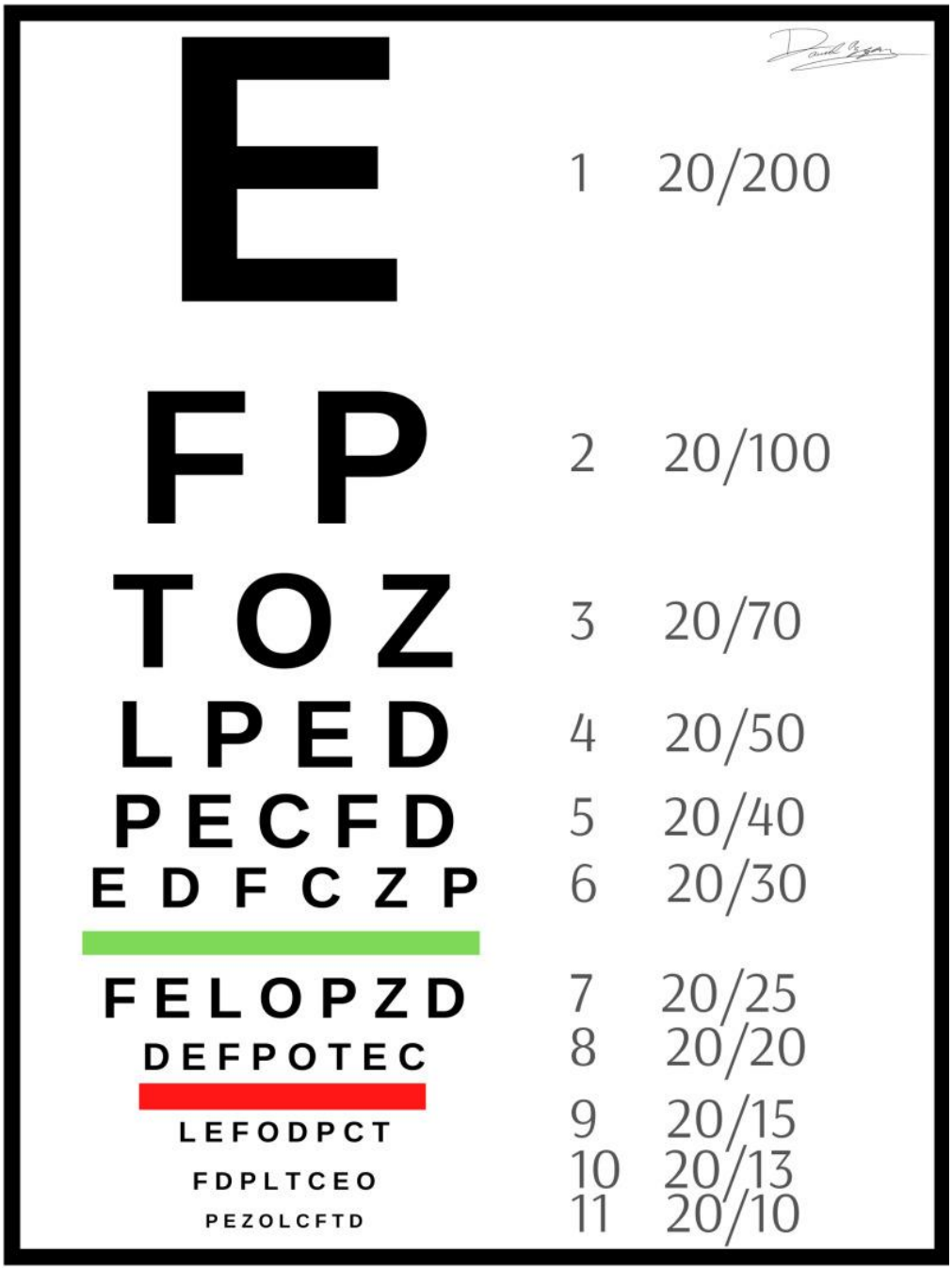
16. Annexes

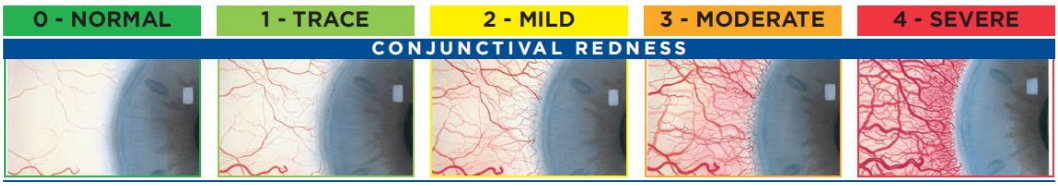
16.1 Cartilla de Snellen



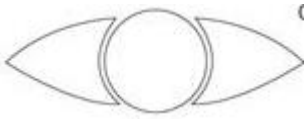
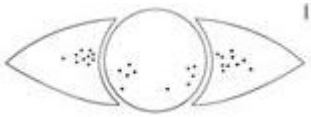
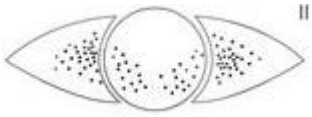
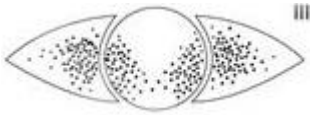
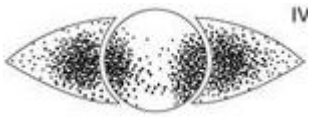
Cartilla de Snellen. Taken from Azzam and Ronquillo, 2021 [65]

The above annex is a representation of the Snellen booklet, it is possible that the booklet designated for the study has variations in format.

16.2. Efron Scale for Conjunctival Hyperemia



16.3 Oxford scale

Panel	Staining pattern	Degree	Criterion	Verbal Description
A		0	Equal to or less than panel A	Absent
B		I	Equal to or less than panel B, greater than panel A	Minimum
C		II	Equal to or less than panel C, greater than panel B	Lightweight
D		III	Equal to or less than panel D, greater than C	Moderate
And		IV	Equal to or less than panel E, greater than panel D	Marked
>E		V	Larger than panel E	Severe

Oxford scale. Staining is represented by the dotted pattern (adapted from Bron AJ et al, 2003) [42]

16.4 Eye Comfort Index (JI)

Índice de Confort Ocular

Ficha de Identificación

No. de estudio: SOPH231-1221/I **Fecha:** _____

Iniciales del sujeto: _____ **No. de sujeto:** _____

Indicaciones:

Este cuestionario fue diseñado para calificar el confort de sus ojos.
Para cada pregunta circule su respuesta

Ejemplo: En la semana pasada, ¿qué tan seguido sus ojos estuvieron rojos?

Nunca						Siempre
0	1	2	3	4	5	6

No existen respuestas correctas o incorrectas. No tome demasiado tiempo en cada pregunta.

1 En la semana pasada, ¿qué tan seguido sus ojos se sintieron *secos* ?

Nunca						Siempre
0	1	2	3	4	5	6

Cuando sus ojos se sentían *secos* , por lo general, ¿qué tan intensa era la sensación?

No he sentido						Severo
0	1	2	3	4	5	6

2 En la semana pasada, ¿qué tan seguido sus ojos se sintieron *arenosos* ?

Nunca						Siempre
0	1	2	3	4	5	6

Cuando sus ojos se sentían *arenosos* , por lo general, ¿qué tan intensa era la sensación?

No he sentido						Severo
0	1	2	3	4	5	6

3 En la semana pasada, ¿qué tan seguido sus ojos sintieron *punzadas* ?

Nunca						Siempre
0	1	2	3	4	5	6

Cuando sus ojos sentían *punzadas* , por lo general, ¿qué tan intensa era la sensación?

No he sentido						Severo
0	1	2	3	4	5	6

4 En la semana pasada, ¿qué tan seguido sus ojos se sintieron *cansados* ?

Nunca						Siempre
0	1	2	3	4	5	6

Cuando sus ojos se sentían *cansados* , por lo general, ¿qué tan intensa era la sensación?

No he sentido						Severo
0	1	2	3	4	5	6

Hoja 1 de 2

Índice de confort ocular

5 En la semana pasada, ¿qué tan seguido sus ojos se sintieron *adoloridos* ?

Nunca						**Siempre**
0	1	2	3	4	5	6

Cuando sus ojos se sentían *adoloridos* , por lo general, ¿qué tan intensa era la sensación?

No he sentido						**Severo**
0	1	2	3	4	5	6

6 En la semana pasada, ¿qué tan seguido sus ojos sintieron *comezón* ?

Nunca						**Siempre**
0	1	2	3	4	5	6

Cuando sus ojos sentían *comezón* , por lo general, ¿qué tan intensa era la sensación?

No he sentido						**Severo**
0	1	2	3	4	5	6

Índice de confort ocular, traducido del Ocular Comfort Index disponible en: http://iovs.arvohournals.org

Hoja 2 de 2

16.5 Fraction to LogMAR conversion table.

LogMAR Value	Snellen (pies)	Snellen (metros)
1.6	20/800	20/240
1.5	20/640	6/190
1.4	20/500	6/150
1.3	20/400	6/120
1.2	20/320	6/96
1.1	20/250	6/75
1.0	20/200	6/60
0.9	20/160	6/48
0.8	20/125	6/38
0.7	20/100	6/30
0.6	20/80	6/24
0.5	20/63	6/19
0.4	20/50	6/15
0.3	20/40	6/12
0.2	20/32	6/9.5
0.1	20/25	6/7.5
0.0	20/20	6/6
-0.1	20/15	6/4.5
-0.2	20/12	6/3.6

Visual acuity equivalence table (adapted from *Ophthalmic & Physiological Optics,* 2016) [69]