

FINAL REPORT

BOVINE CORNEAL OPACITY AND PERMEABILITY ASSAY

Laboratory Study Number:

20AA02.350000

Study Completion Date:

18 February 2020

Authors:

Allison Hilberer, M.S., DABT
Kayla Cantrell, B.S.
Samantha Weiger, B.S.

Sponsor

Banixx
132 Aqua Shed Ct.
Abendeen, NC 28315

Performing Laboratory:

Institute for In Vitro Sciences, Inc.
30 W. Watkins Mill Road, Suite 100
Gaithersburg, MD 20878

Laboratory Project Number:

10874

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SIGNATURE PAGE

Initiation Date: 20 January 2020

Laboratory Start Date: 22 January 2020

Laboratory Completion Date: 22 January 2020

Completion Date: 18 February 2020

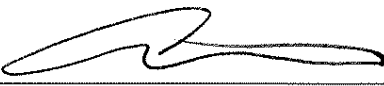
Sponsor's Representative: Bob Demeulemester

Testing Facility: Institute for In Vitro Sciences, Inc.
30 W. Watkins Mill Road, Suite 100
Gaithersburg, MD 20878

Archive Location: Institute for In Vitro Sciences, Inc.
Gaithersburg, MD 20878

Director, Laboratory Services: Gertrude-Emilia Costin, Ph.D., M.B.A., ATS, ERT

Study Director:



Allison Hilberer, M.S., DABT *18 February 2020*
Date

TEST ARTICLE RECEIPT

IIVS Test Article Number	Sponsor's Designation	Physical Description	Receipt Date	Storage Conditions*
20AA02	Banixx-BJG451	clear colorless non-viscous liquid	13 January 2020	15 to 30 °C (Room Temp)

* - Protected from exposure to light

INTRODUCTION

The Bovine Corneal Opacity and Permeability Assay (BCOP) was used to assess the potential ocular irritancy of the test article to isolated bovine corneas. Bovine corneas, obtained as a byproduct from freshly slaughtered animals, were mounted in special holders and exposed to the test article. An *In Vitro* Score was determined for the test article based on the induction of opacity and permeability (to fluorescein) in the isolated bovine corneas. The methods and procedures used in this assay were consistent with OECD Test Guideline 437: Bovine Corneal Opacity and Permeability Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage (2017)¹.

MATERIALS AND METHODS

The assay procedures were performed as outlined in the study protocol ([See Appendix A](#)). The test article was tested according to the Method A described in the study protocol.

DEVIATIONS

There were no deviations from the study protocol during the conduct of this study.

¹ OECD (2017), *Test No. 437: Bovine Corneal Opacity and Permeability Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage*, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing.

RESULTS AND DISCUSSION

Test Article & Assay Control Preparation

The test article was administered to the test system without dilution (neat).

The positive control (100% ethanol) and the negative control (sterile, deionized water) were tested concurrently.

Exposure Times

Three corneas were incubated in the presence of the test article at 32 ± 1 °C for 10 minutes. Three corneas were incubated in the presence of each control at 32 ± 1 °C for 10 minutes.

Evaluation of Test Results

[Table 1](#) summarizes the results of the BCOP assay for the test article and positive control. The analyzed data are presented in [Appendix B](#). The following prediction models were used to evaluate the ocular irritancy potential as described by Sina et al. (1995)² for non-regulatory purposes or as described in OECD TG 437 (2017).

The prediction model according to Sina et. al based on a wide range of materials (*note: While this classification system provides a good initial guide to interpretation of these in vitro data, these specific ranges may not be applicable to all classes of materials or other exposure times. Whenever possible, results should be compared to “benchmark” materials tested under similar exposure conditions.*):

<i>In Vitro</i> Score	Sina et al Prediction
≤ 25	Mild irritant
> 25; ≤ 55	Moderate irritant
> 55	Severe irritant

² Sina, J.F., Galer, D.M., Sussman, R.G., Gautheron, P.D., Sargent, E.V., Leong, B., Shah, P.V., Curren, R.D., and Miller, K. (1995) A collaborative evaluation of seven alternatives to the Draize eye irritation test using pharmaceutical intermediates. **Fundamental and Applied Toxicology** 26:20-31.

Prediction model outlined in OECD TG 437:

<i>In Vitro</i> Irritation Score (IVIS)	UN GHS
≤ 3	No Category
> 3; ≤ 55	No prediction can be made*
> 55	Category 1

* - Additional testing would be required for a definitive classification of ocular irritation potential according to GHS

Criteria for a Valid Test.

The BCOP assay was accepted when the positive control ethanol produced an *In Vitro* Score that fell within 2 standard deviations of the historical mean. The current acceptance range for ethanol is 38.0-63.4.

Summary

The test article produced an *In Vitro* Score of 0.0. According to the prediction model presented by Sina et al. (1995), the test article would be considered to have mild ocular irritation potential (i.e., *In Vitro* Score ≤25). According to OECD TG 437, the test article was considered GHS No Category for eye irritation potential (i.e., *In Vitro* Score ≤3.0).

Table 1

BCOP Summary Results

IIVS Test Article Number	Sponsor's Designation	Conc.	Exposure Time	Opacity Value	OD₄₉₀ Value	In Vitro Score	pH
20AA02	Banixx-BJG451	Neat	10 minutes	0.0	-0.003	0.0	1.0
Positive Control	Ethanol	Neat	10 minutes	27.7	0.897	41.1	NA

NA – Not Applicable

APPENDIX A (Protocol, Protocol Attachment 1 & Protocol Amendment I)

BOVINE CORNEAL OPACITY AND PERMEABILITY ASSAY

1.0 PURPOSE

The purpose of this study is to evaluate the potential ocular irritancy/toxicity of a test article as measured by the test article's ability to induce opacity and permeability to fluorescein in an isolated bovine cornea.

2.0 SPONSOR

- 2.1 Name: Bannix
- 2.2 Address: 132 Aqua Shed Ct.
Abendeen, NC 28315
- 2.3 Representative: Bob Demeulemester

3.0 IDENTIFICATION OF TEST ARTICLE(S) AND ASSAY CONTROLS

- 3.1 Test Article(s): See Protocol Attachment 1
- 3.2 Assay Controls: Positive: Ethanol (CAS #64-17-5) Neat (liquid test articles)
- Imidazole (CAS #288-32-4) 20% (w/v) in Complete MEM (solid test articles)
- Negative: Sterile deionized water or appropriate solvent

3.3 Determination of Strength, Purity, etc.

- 3.3.1 For GLP studies, the Institute for In Vitro Sciences, Inc. (IIVS) will attempt to secure documentation of the analytical purity and composition of the test article and the stability and strength of the dosing solutions from the Sponsor. If the Sponsor is unable to provide such information, the final report will be generated with an exception noted in the Statement of Compliance.
- 3.3.2 IIVS will be responsible for the documentation of the analytical purity and composition of the positive and negative controls used. This may be accomplished by maintaining a certificate of analysis from the supplier.

4.0 TESTING FACILITY AND KEY PERSONNEL

- 4.1 Name: Institute for In Vitro Sciences, Inc.
- 4.2 Address: 30 W. Watkins Mill Road, Suite 100
Gaithersburg, MD 20878
- 4.3 Study Director: Allison Hilberer, M.S., DABT

5.0 TEST SCHEDULE

- 5.1 Proposed Experimental Initiation Date: 23 January 2020
- 5.2 Proposed Experimental Completion Date: 7 February 2020
- 5.3 Proposed Report Date: 20 March 2020

6.0 TEST SYSTEM

The test system (target tissue) is the isolated bovine cornea obtained as a by-product from freshly slaughtered animals. The procedures for preparing and handling the test system were developed by Gautheron et al. (1992). The assay measures two important components which are predictive of eye irritation: corneal opacity and permeability. Each cornea holder will be uniquely identified with a number written in permanent marker, on both the anterior and posterior chambers. The treatment of each cornea will be identified with the test article number (or control) written in permanent marker on colored tape, affixed to each holder.

7.0 EXPERIMENTAL DESIGN AND METHODOLOGY

The methods for the Bovine Corneal Opacity and Permeability Assay presented herein are based on the procedures described in the OECD test guideline "*Bovine Corneal Opacity and Permeability Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage*" (OECD TG 437, adopted 9 October 2017).

Liquid test articles will be tested neat unless otherwise directed by the Sponsor. If the liquid test article is to be diluted, the study may begin with a solubility or miscibility test in sterile, deionized water or appropriate solvent designated by the Sponsor. Solid test articles will be diluted in sterile deionized water unless otherwise directed by the Sponsor. The pH of each neat (liquid) test article or diluted test article will be determined, if possible. Three corneas treated with sterile deionized water will serve as the negative control. Three corneas will be exposed to the positive control. The number of corneas treated with each neat test article or test article solution/suspension is specified in Protocol Attachment 1.

One of two treatment methods will be used depending on the physical state and chemical characteristics (liquid or surfactant versus non-surfactant solid) of the test article.

Changes in opacity and permeability to fluorescein will be measured and used to assess the relative potential for ocular irritancy of the test article(s).

7.1 Reagents:

- 7.1.1 Hanks' Balanced Salt Solution with Ca⁺⁺ and Mg⁺⁺ (containing penicillin/streptomycin, sodium bicarbonate, and L-glutamine) (HBSS)
- 7.1.2 Minimum Essential Medium (EMEM) without phenol red supplemented to contain 1% Fetal Bovine Serum (FBS) and 2 mM L-glutamine (Complete MEM without phenol red)
- 7.1.3 Minimum Essential Medium (EMEM) containing phenol red supplemented to contain 1% FBS and 2 mM L-glutamine (Complete MEM containing phenol red)
- 7.1.4 Sodium Fluorescein — diluted in Dulbecco's Phosphate Buffered Solution containing Ca⁺⁺ and Mg⁺⁺
- 7.1.5 Sterile deionized water

7.2 Environmental Conditions

Throughout this protocol, ranges for test material and test system exposure or incubation conditions (e.g., temperature, humidity, CO₂) are presented. These ranges describe the equipment performance specifications under static conditions (i.e., in the absence of frequent opening of equipment doors, accessing chambers, changing loads, etc.), as presented in the relevant equipment SOPs.

7.3 Bovine Eyes

Bovine eyes will be obtained from the abattoir of J.W. TREUTH & SONS, Inc., Baltimore, MD. The eyes will be excised by an abattoir employee (as soon after slaughter as possible) and held in HBSS on ice. Once the required number of eyes has been obtained, the eyes will be transported to IIVS. Immediately upon receipt of the eyes into the laboratory, preparation of the corneas will be initiated.

7.4 Preparation of Corneas

All eyes will be carefully examined for defects (opacity, scratches, pigmentation, etc.) and those exhibiting defects discarded. The tissue surrounding the eyeball will be carefully pulled away and the cornea will be excised leaving a 2 to 3 mm rim of sclera. The isolated corneas will be stored in a petri dish containing HBSS prior to mounting. Corneas will then be mounted in the corneal holders with the endothelial side against the O-ring of the posterior chamber. The anterior chamber will then be positioned on top of the cornea and tightened with screws. The chambers of the corneal holder will then be filled with Complete MEM

(without phenol red). The posterior chamber will always be filled first. The corneas will be incubated for the minimum of one hour at 32 ± 1 °C.

7.5 Sample Preparations

When appropriate, test articles will be diluted or suspended in either sterile deionized water or other Sponsor-directed solvent. Samples will be diluted on a w/v basis, unless otherwise specified by the Sponsor.

The stability of the test article under the storage conditions at the testing facility and under the actual experimental conditions will not be determined by Institute for In Vitro Sciences, Inc. (IIVS).

7.6 Treatment of Corneas

At the end of the 1-hour incubation period, the medium will be removed from both chambers and replaced with fresh Complete MEM (without phenol red). An initial opacity measurement will be performed on each of the corneas using the OP-KIT (Electro Design) opacitometer. The opacity of each cornea (including the negative control corneas) will be read against an air-filled chamber and recorded. Corneas that have an initial opacity reading greater than 7 will not be used and will be discarded. The medium will be removed from the anterior chamber and replaced with the test article, negative control, or positive control.

Protocol Attachment 1 will provide the test article designation(s), any preparation (including dilution and handling of the test material), the method to be used (if applicable), the length of the treatment, the post-treatment incubation time(s) and the applicable regulations to be followed.

7.6.1 Method A

Liquids will generally be tested neat (undiluted), unless the Sponsor requests a specific dilution. Surfactants (either solids or liquids) will generally be tested at a 10% concentration in sterile deionized water unless otherwise directed by the Sponsor.

Seven hundred and fifty microliters of test substance (test article, negative control or positive control) will be introduced into the anterior chamber. The holder will be slightly rotated (with the corneas maintained in a horizontal position) to ensure uniform distribution of the test substance over the cornea. Alternatively, the test material may be applied as a spray to cover the corneal surface. Spray application will be used only when directed by the Sponsor and will follow the specific procedure indicated. The test article will be incubated at the exposure time identified in Protocol Attachment 1. Exposure times of 3 minutes or less will be incubated at room temperature. Exposure times > 3 minutes will be incubated at 32 ± 1 °C. The positive control will be incubated at 32 ± 1 °C for 10 minutes. The negative control treated corneas will be incubated at

32 ± 1 °C for 10 minutes. On occasion, the negative control exposure time may be selected to fit the longest test article exposure time of a test article run concurrently, but from an independent study. The test substance will then be removed and the epithelium will be washed at least 3 times (or until no visual evidence of test substance can be observed) with Complete MEM (containing phenol red). Once the media is free of test substance, the corneas will be given a final rinse with Complete MEM (without phenol red). If the test article cannot be removed from the cornea a note will be documented in the raw data record. The anterior chamber will then be refilled with fresh Complete MEM without phenol red and an opacity measurement will be performed. The corneas will then be incubated for approximately 2 hours at 32 ± 1 °C. For test substances and negative control treated corneas with exposure times greater than 10 minutes, the post-exposure period will be adjusted by subtracting the test article exposure from 2 hours. At the completion of the post-exposure incubation period, a second measure of opacity will be performed (final opacity). The values obtained at this second measurement will be used in calculating the corneal opacity.

7.6.2 Method B

Solid materials will generally be tested as a 20% dilution (w/v) in sterile deionized water (or Sponsor directed solvent). Different concentrations may be evaluated at the Sponsor's request.

Seven hundred and fifty microliters of test substance (test article, negative control or positive control) will be introduced into the anterior chamber. In some cases, the 20% (w/v) suspension may not be pipettable (e.g., test article floating in liquid), and a positive displacement pipet cannot be used. In those cases, a dosing spoon of the same approximate diameter of the exposed epithelium may be used to dose the corneas. Although it is understood that a 750 µL dose cannot be achieved, the corneas should be completely covered with the test article. The holder will be slightly rotated (with the corneas maintained in a horizontal position) to ensure uniform distribution of the test substance over the cornea. The corneas will be incubated in a horizontal position at 32 ± 1 °C for approximately 4 hours or as specified by the Sponsor. The test substance will then be removed and the epithelium washed at least 3 times (or until no visual evidence of test substance can be observed) with Complete MEM (containing phenol red). Once the media is free of test substance, the corneas will be given a final rinse with Complete MEM (without phenol red). If the test article cannot be removed from the cornea a note will be recorded in the raw data record. The anterior and the posterior chambers will then be refilled with fresh Complete MEM (without phenol red), and an opacity measurement performed immediately (without any further incubation) (final opacity).

7.7 Opacity Measurement

The opacitometer will determine the difference in the light transmission between each treated or control cornea and an air-filled chamber, and a numerical opacity value (arbitrary unit) will be displayed and recorded.

7.8 Permeability Determinations

7.8.1 Method A

After the second opacity measurement is performed, the medium will be removed from both chambers of the holder. The posterior chamber will be refilled with fresh Complete MEM (without phenol red). One milliliter of a 4 mg/mL sodium fluorescein solution will be added to the anterior chamber.

7.8.2 Method B

After the opacity measurement is performed, the medium will be removed from the anterior chamber only and replaced with 1 mL of a 5 mg/mL sodium fluorescein solution.

For Method A and B, after the addition of the fluorescein solution to the anterior chamber, the corneas will be incubated in a horizontal position for approximately 90 minutes at 32 ± 1 °C. The medium from the posterior chamber will be removed at the completion of the incubation period, and 360 µL will be transferred to the appropriate wells of a labeled 96-well plate. Three hundred and sixty microliters of fresh Complete MEM (without phenol red) will be added to the wells designated as blanks. The optical density at 490 nm (OD_{490}) will be determined using a spectrophotometer. Samples reading 1.500 and above (OD_{490}) will be diluted to bring the reading within the linear range of the platereader and the plate read again.

8.0 CRITERIA FOR DETERMINATION OF A VALID TEST

The test will be accepted if the positive control produces an *In Vitro* Score that falls within two standard deviations of the historical mean.

9.0 EVALUATION OF TEST RESULTS

The change in opacity for each cornea (including the negative control corneas) will be calculated by subtracting the initial opacity reading from the final opacity reading. These values will then be corrected by subtracting from each the average change in opacity observed for the negative control corneas. The mean opacity value for each treatment will be calculated by averaging the corrected opacity values of each cornea for a given treatment.

The mean OD₄₉₀ for the blank wells will be calculated. The mean blank OD₄₉₀ will be subtracted from the OD₄₉₀ of each well (corrected OD₄₉₀). Any dilutions that are made to bring the OD₄₉₀ values into the linear range of the platereader will have each diluted OD₄₉₀ value multiplied by the dilution factor. The final corrected OD₄₉₀ of the test article(s) and the positive control will be calculated by subtracting the average corrected OD₄₉₀ of the negative control corneas from the corrected OD₄₉₀ value of each treated cornea:

$$\text{Final Corrected OD}_{490} = (\text{OD}_{490} - \text{Mean Blank OD}_{490}) - \text{Average Corrected Negative Control OD}_{490}$$

The mean OD₄₉₀ value of each treatment group will be calculated by averaging the final corrected OD₄₉₀ values of the treated corneas for that treatment condition. Although the algorithms discussed are performed to calculate the final endpoint analysis at the treatment group level, the same calculations can be applied to the individual replicates.

9.1 *In Vitro* Score Calculation

The following formula will be used to determine the *In Vitro* Irritation Score:

$$\text{In Vitro Score} = \text{Mean Opacity Value} + (15 \times \text{Mean OD}_{490} \text{ Value})$$

9.2 Data Interpretation

9.2.1 Data Interpretation (OECD Test Guideline 437)

For regulatory purposes, the *In Vitro* Irritation Score (IVIS) cut-off values for identifying test chemicals as inducing serious eye damage (UN GHS Category 1) and test chemicals not requiring classification for eye irritation or serious eye damage (UN GHS No Category) are found in the table below (OECD 437, adopted 9 October 2017). This guidance on categorization applies only to test article(s) evaluated using the appropriate standard protocols as described in OECD 437.

IVIS	UN GHS
≤ 3	No Category
> 3; ≤ 55	No prediction can be made
> 55	Category 1

9.2.2 Data Interpretation (Sina Scale)

For non-regulatory purposes, the following classification system was established by Sina et al. based on studies with a wide range of test materials. While this classification system provides a good initial guide to interpretation of these *in vitro* data, these specific ranges may not be applicable to all classes of materials or other exposure times. Whenever possible, results should be compared to “benchmark” materials tested under similar exposure conditions.

In Vitro Irritation Score:

≤ 25	=	mild irritant
from 25.1 to 55	=	moderate irritant
from 55.1 and above	=	severe irritant

10.0 REPORT

A report of this study will be prepared by the Testing Laboratory and will accurately describe all methods used for generation and analysis of the data. A summary will be presented for each treatment group. The report will also include a discussion of results. A copy of the protocol used for the study and any significant deviation(s) from the protocol will appear as a part of the final report.

11.0 RECORDS AND ARCHIVES

A separate working notebook will be used to record the materials and procedures used to perform this study. Upon completion of the final report, all raw data, reports and specimens will be retained in the archives for a period of either a) 5 years, b) the length of time specified in the contract terms and conditions, or c) as long as the quality of the preparation affords evaluation, whichever is applicable.

12.0 TEST MATERIAL RETENTION

Unless indicated otherwise, all test articles provided by the Sponsor will be retained for 6 months after completion of the final report. These test articles may be disposed after this 6 month retention period according to IIVS SOP. Unless indicated otherwise, dose dilutions used for testing or analysis before or during the course of the assay will be discarded after testing.

13.0 PROTOCOL AMENDMENTS

When it becomes necessary to change the approved protocol for a specific study, the change and the reason for it should be put in writing and signed by the Study Director as soon as practical. When the change may impact the study design and/or execution, verbal agreement to make this change should be made between the Study Director and Sponsor. This document is then provided to the Sponsor and is attached to the protocol as an amendment.

14.0 REFERENCES

Gautheron, P., Dukic, M., Alix, D., and Sina, J.F. (1992) Bovine Corneal Opacity and Permeability Test: An *In Vitro* Assay of Ocular Irritancy. **Fundamental and Applied Toxicology** 18:442-449.

OECD (2017), *Test No. 437: Bovine Corneal Opacity and Permeability Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage*, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing. doi: 10.1787/9789264203846-en

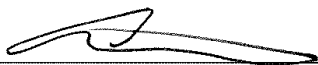
Sina, J.F., Galer, D.M., Sussman, R.G., Gautheron, P.D., Sargent, E.V., Leong, B., Shah, P.V., Curren, R.D., and Miller, K. (1995) A collaborative evaluation of seven alternatives to the Draize eye irritation test using pharmaceutical intermediates. **Fundamental and Applied Toxicology** 26:20-31.

15.0 APPROVAL

Bob DeMeulemester

SPONSOR REPRESENTATIVE

Bob Demeulemester



IIVS STUDY DIRECTOR

Allison Hilberer, M.S., DABT

1/20/2020

DATE

20 January 2020

DATE

PROTOCOL ATTACHMENT 1

IIVS Test Article Designation	Sponsor Designation
20AA02	Bannixx-BJG451

Test Article Preparation: The test article will be tested without dilution (neat).

Corneas Treated per Test Article: 3
 4-5

Test Method: Method A
 Method B

Test Article Exposure Time 10 minutes

Test Article Post-Exposure Incubation Time: 120 minutes

REGULATORY REQUIREMENTS:

Will this study be conducted according to GLPs? YES or NO

If YES, please indicate which agency(ies) guidelines are to be followed:

FDA | OECD | Other: _____

EPA TSCA (40 CFR 792) | EPA FIFRA (40 CFR 160)

IIVS Study No.:20AA02.350000
IIVS Project No.: 10874

PROTOCOL AMENDMENT I

SPONSOR:	Banixx
IIVS STUDY NO.:	20AA02.350000

AMENDMENT:

1) Location: Protocol, Page 1, Section 2.1

Amendment: Update: Bannix
To: Banixx

Reason: protocol generation error

2) Location: Protocol Attachment 1, Table, Sponsor Designation

Amendment: Update: Bannix-BJG451
To: Banixx-BJG451

Reason: protocol generation error

APPROVAL:



STUDY DIRECTOR

4 February 2020
DATE

APPENDIX B (Analyzed Data)

BOVINE CORNEAL OPACITY AND PERMEABILITY ASSAY

OPACITY SCORE

<u>TA #</u>	<u>CORNEA #</u>	<u>INITIAL</u>	<u>FINAL</u>	<u>CHANGE</u>	<u>CORRECTED</u>	<u>AVG</u>	<u>STDEV</u>
20AA02	25	0	0	0	0.0		
Neat	26	0	0	0	0.0		
10 minutes	28	0	0	0	0.0	0.0	0.0
Neg. Control	1	0	0	0	NA		
Sterile, DI water	4	0	0	0	NA		
10 minutes	6	0	0	0	NA	0.0	
Pos. Control	8	0	27	27	27.0		
Ethanol	9	0	27	27	27.0		
10 minutes	11	0	29	29	29.0	27.7	1.2

NA - Not Applicable

PERMEABILITY SCORE

**Neg. Control
Sterile, DI water
10 minutes**

Cornea #	OD490
1	0.005
4	0.007
6	0.004

Avg.	0.005

**Pos. Control
Ethanol
10 minutes**

Cornea #	OD490	Dilution Factor	Corrected OD490
8	0.876	1	0.871
9	0.942	1	0.937
11	0.889	1	0.884

Avg. =			0.897
STDEV =			0.035

**20AA02
Neat
10 minutes**

Cornea #	OD490	Dilution Factor	Corrected OD490
25	0.003	1	-0.002
26	0.001	1	-0.004
28	0.004	1	-0.001

Avg. =			-0.003
STDEV =			0.002

IN VITRO SCORE

In Vitro Score = Mean Opacity Value + (15 x Mean OD490)

Test Article	Concentration	Exposure Period	Mean Opacity	Mean OD490	In vitro Score
20AA02	Neat	10 minutes	0.0	-0.003	0.0
Ethanol	Neat	10 minutes	27.7	0.897	41.1