



瑞德生物科技有限公司
MASTER LABORATORY CO.,LTD.

Ocular Irritation Study in Rabbits

Master Laboratory Co., Ltd. Animal Laboratory

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**0.5% Virol-Oxy
Ocular Irritation Study in Rabbits
STUDY REPORT**



Sponsor: Watch Water (S) Pte Ltd

Testing Institution: Master Laboratory Co., Ltd.

June 2020



FINAL REPORT

Report No.: MSA-202006-184-T05

Report No. : MSA-202005-184-T05

IACUC No. : MSA202004-05

Experimental starting date: 06.02.2020

Test article registration date: 06.05.2020

Animal in-housing: 06.02.2020

Extraction of test article: 06.06.2020

Test article administration date: 06.09.2020

Observation of ocular irritation reaction : 06.09.2020-06.12.2020

Study Announcement

1. The study report is valid for the test article used only, and shall not be partly recopied or extracted for another object.
2. The study report is invalid without the endorsement of Master Laboratory Co., Ltd.



SIGNATURE OF STUDY PERSONNEL

Experiment executor: Bo Han Huang

Study Director

Jhen Ru Shih
Jhen Ru Shih

06.16.2020
Date

Facility Management

Alan Hsieh
Alan Hsieh

06.16.2020
Date



STUDY DIRECTOR COMPLIANCE STATEMENT

The study met with the technical requirements of the protocol, and all applicable guidance and regulations, which included the Good Laboratory Practice for Non-clinical Laboratory Studies (FDA, 21 CFR, Part 58, 2019), Good Laboratory Practice for Non-clinical Laboratory Studies (Ministry of Health and Welfare, R.O.C., 3rd ed., 2006) and the General Requirements for the Competence of Testing and Calibration Laboratories (ISO/IEC 17025:2017). Besides, there was no deviation from the approved study protocol and no adverse problems that would affect the integrity of the results or the interpretation of our conclusion. The test article is a proprietary product of the sponsor, therefore the sponsor will be responsible for the requirements listed under “0.5% Virol-Oxy” of the GLP regulation (21CFR P.58, FDA).

Study Director

Jhen Ru Shih
Jhen Ru Shih

06.16.2020
Date



QUALITY ASSURANCE STATEMENT

To comply with the “Good Laboratory Practice for Nonclinical Laboratory Study”, Quality Assurance Unit has audited the facility, equipment, personnel, test methods, raw data, and records regularly.

The study report has been reviewed and approved. The experiments were conducted according to the protocol. All original records, raw data, and documents are truthfully transferred and addressed in the results of this report.

Inspection record:

Inspection Contents	Date of inspection
Before the test (test execution protocol, requisitions, contracts).....	06.02.2020
Test (test substance data sheet, animal quarantine, standard operating procedures).....	06.09.2020
After the test (complete the original data, report reviews).....	06.16.2020

Quality Assurance unit in charge

Ying Chun Chen
Ying Chun Chen

06-16-2020
Date



Contract Research Organization and Sponsor Information

1. CRO:

- a. Title: Master Laboratory Co., Ltd.-Animal laboratory
- b. Address: 3F, No. 221, Sec. 1, Zhongxing Rd., Zhudong Township, Hsinchu County 31053, Taiwan, R.O.C.

2. Sponsor:

- a. Title: Watch Water (S) Pte Ltd
- b. Address: 25 International Business Park #01-26/29 German Centre Singapore 609916



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SUMMARY

The present study was performed in compliance with ISO 10993-10:2010 to investigate the acute ocular irritation response of “0.5% Virol-Oxy” extract on the ocular of New Zealand White Rabbits. The test article extracts and the control solution (0.9% saline and cottonseed oil) were dropped on the right eye and left eye respectively. At the time points of 1st, 24th, 48th and 72nd hr thereafter were observed. There were no significant clinical abnormality and gross reaction in either the control or treatment group (Table 1, Figure 1-8 and Appendix 1). All animals were in overall good health over the course of the study. Therefore, a single instillation of 0.1 ml of test article extract did not cause acute ocular irritation in New Zealand White Rabbits.



INTRODUCTION

This study was performed in compliance with ISO 10993-10:2010 guideline (Test for irritation and skin sensitization, Annex B (informative) special irritation tests) for irritation study. The study was to evaluate the possibility of local irritant reaction after a single instillation of test article extract on the ocular of New Zealand White Rabbits.

MATERIALS AND METHODS

1. Animals

1.1. Species/Strain: New Zealand White Rabbit

1.2. Resource: Hui Jun

1.3. Body weights (gender): >2 kg (Male)

1.4. Quarantine/acclimation (MSAT-SOP-AM-001): Animals were subjected to be quarantined and acclimated before treatment. Veterinarian ensured the animal health status before the treatment.

1.5. Reasons chosen for animal experimentation: New Zealand White Rabbits were proven to be suitable for ocular irritation studies, and they were widely used in single dose ocular irritation studies.

1.6. Grouping

Polar Group	Control (left ocular)	Treatment (right ocular)
Number of animals		3
Treated article	0.9% saline	0.5% Virol-Oxy extract by 0.9% saline

Remark: The control solution and the test article extract were applied on the same rabbit.



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Non-polar Group	Control (left ocular)	Treatment (right ocular)
Number of animals		3
Treated article	Cottonseed oil	0.5% Virol-Oxy extract by cottonseed oil

Remark: The control solution and the test article extract were applied on the same rabbit.

2. Feeding and care (MSAT-SOP-AM-001)

2.1. Housing: Rabbit room

2.2. Environment (SOP: Temperature: $19 \pm 3^{\circ}\text{C}$; Humidity: $50 \pm 20\%$)

a. Temperature: $20.8\text{-}21.8^{\circ}\text{C}$

b. Humidity: 54-58%

c. Light Cycle: 12 hours light and 12 hours dark

2.3. Cage and animal no.

a. Quarantine/acclimation: 1 rabbit/cage

b. Study period: 1 rabbit/cage

2.4. Feed

a. Name: Prolab Rabbit Diet

b. Brand: Lab Diet, U.S.A.

c. Way to supply: *ad libitum*

d. Source: PMI Nutrition International, U.S.A.

2.5. Drinking water

a. Sort: RO Water

b. Way to supply: *ad libitum*

3. Individual and group identification

3.1. Individual identification: Tested animals were identified by ear-marking.



3.2. Group identification: Cages were properly labeled for identification including the Study Title/No., Administration, Observation Period, Room No., Cage No., Quantity/cage, Species, Strain, Gender, In House Date, In House Age, Animal ID No., Keeper and Deputy.

4. Test article and Control

4.1. Test article: 0.5% Virol-Oxy

4.2. 0.9% saline: CHI SHENG CHEMICAL CORPORATION, Lot. N6012;
Solutio Natrii Chloridi Isotonica 0.9% 500 ml

4.3. Cottonseed oil: SIGMA-ALDRICH, Co., Lot. MKCB9547;
CAS No.: 8001-29-4

5. Administration of test article and control solution

5.1. Preparation (MSAT-SOP-GE-014) According to ISO 10993-12:2012 guideline.

a. Polar preparation: Measured the weight of test article, and immersed it in 0.9% saline for 72 ± 2 hr at $50 \pm 2^\circ\text{C}$ with constant agitation (100 rpm). The weight ratio of test article/0.9% saline was approximately 0.2 g/ml. After extraction, the extract solution was used immediately; the appearance of test article extract was clear and colorless without particulates present.

b. Non-polar preparation: Measured the weight of test article, and immersed it in cottonseed oil for 72 ± 2 hr at $50 \pm 2^\circ\text{C}$ with constant agitation (100 rpm). The weight ratio of test article/cottonseed oil was approximately 0.2 g/ml. After extraction, the extract solution was used immediately; the appearance of test article extract was clear and colorless without particulates present.

5.2. Method, route and frequency of administration: A single dose of test article extract and control solution were dropped on right and left eye.



5.3. Volume of administration: Test article extract or control solution was 0.1 ml.

6. Procedure (MSAT-SOP-ME-001)

6.1. Prior to study, check the ocular.

6.2. Test article administration: On the treatment day, 0.1 ml of the test article was dropped on right eye.

6.3. Control solution administration: On the treatment day, 0.1 ml of 0.9% saline or cottonseed oil was dropped on left eye.

7. Animal observations and items for examination (MSAT-SOP-ME-001)

7.1. Irritant reaction evaluation: At the time points of 1st, 24th, 48th and 72nd hr were observed of the test article extract and control solution. If the test article caused the irritant reaction at 72nd hr, the observation and recordings should be continued until the 21th day.

7.2. Determination of dermal reaction: After a single dose treatment, the ocular responses at 1st, 24th, 48th and 72nd hr were checked and evaluated.

7.3. Evaluation of results:

- a. If the treated eye in more than one animal shows a positive result (footnoted grades in Appendix 2) at any of the observations, then the material is considered an eye irritant and further testing is not required.
- b. If only one of three treated eyes shows a mild or moderate reactions are equivocal, treat further animals.
- c. When further animals have been treated, the test material is considered to be an eye irritant if more than half of the eyes treated in the test group exhibit a positive result (footnoted grades in Appendix 2) at any stage of the observation. A severe reaction in only one animal is considered sufficient to label the material as an eye irritant.



RESULTS

The study was performed in compliance with ISO 10993-10:2010 to investigate the response of ocular irritation of “0.5% Virol-Oxy” extract was dropped on the right eye, and the control solution on left eye. At the time points of 1st, 24th, 48th and 72nd hour thereafter were observed. The results showed that there were no slight vessels definitely on redness on polar or non-polar group, and there were no significant clinical abnormality and gross reaction in either the control or other treatment group (Table1, Figure1-8 and Appendix 1). All animals were in overall good health over the course of the study. Therefore, a single instillation of 0.1 ml of test article extract did not cause ocular irritation in New Zealand White Rabbits.

CONCLUSION

Under the results of this study, a single instillation with 0.1 ml of test article “0.5% Virol-Oxy” extract did not cause observable irritation in New Zealand White Rabbits. The response of test article extract was considered as negligible in rabbits.



REFERENCES

1. Good Laboratory Practice for Nonclinical Laboratory Studies (2006) Food and Drug Administration, the Executive Yuan.
2. Guideline for the Nonclinical Pharmacology/Toxicology Studies Medicinal Products Applications (2013) Food and Drug Administration, the Executive Yuan.
3. Good Laboratory Practice for Nonclinical Laboratory Studies. Title 21 of the U.S. Code of Federal Regulations, Part 58 (2015) United States Food and Drug Administration.
4. Biological evaluation of medical devices-part 10: Test for irritation and skin sensitization, Annex B (informative) special irritation tests ISO 10993 (2010).
5. Biological evaluation of medical devices-Part 12: Sample preparation and reference materials ISO 10993 (2012).
6. Good Laboratory Practice for Non-clinical Laboratory Studies (Ministry of Health and Welfare, R.O.C., 3rd ed., 2006).
7. ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories.



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Table 1. Incidence of Clinical Observation in Rabbits

Polar Group	Control (left eye)	Treatment (right eye)
Number of animals	3	
Treated article	0.9% saline	0.5% Virol-Oxy extract by 0.9% saline
Cornea	0/3	0/3
Iris	0/3	0/3
Conjunctivae	0/3	0/3

Remark : The control solution and test article were dropped on the same rabbit.

n/n : No. of rabbits with clinical signs/No. of rabbits per group

Non-polar Group	Control (left eye)	Treatment (right eye)
Number of animals	3	
Treated article	Cottonseed oil	0.5% Virol-Oxy extract by cottonseed oil
Cornea	0/3	0/3
Iris	0/3	0/3
Conjunctivae	0/3	0/3

Remark : The control solution and test article were dropped on the same rabbit.

n/n : No. of rabbits with clinical signs/No. of rabbits per group



**Left eye
(control)**



**Right eye
(treatment)**

Figure 1. Observation at the 1st hr of Administration (polar group)



**Left eye
(control)**



**Right eye
(treatment)**

Figure 2. Observation at the 24th hr of Administration (polar group)



**Left eye
(control)**



**Right eye
(treatment)**

Figure 3. Observation at the 48th hr of Administration (polar group)



**Left eye
(control)**



**Right eye
(treatment)**

Figure 4. Observation at the 72nd hr of Administration (polar group)



**Left eye
(control)**



**Right eye
(treatment)**

Figure 5. Observation at the 1st hr of Administration (non-polar group)



**Left eye
(control)**



**Right eye
(treatment)**

Figure 6. Observation at the 24th hr of Administration (non-polar group)



**Left eye
(control)**



**Right eye
(treatment)**

Figure 7. Observation at the 48th hr of Administration (non-polar group)



**Left eye
(control)**



**Right eye
(treatment)**

Figure 8. Observation at the 72nd hr of Administration (non-polar group)



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Appendix 1-1. Individual Animal Grade in Clinical Observation of Rabbits

Applied Regions (Ocular) Polar-group	Treated article	Gender	Animal No.	Items for Grading	Clinical Observation (time point/h)			
					1	24	48	72
Right eye	Treatment group 0.5% Virol-Oxy extract by "0.9% saline"	Male	184-1001	Cornea	0	0	0	0
				Iris	0	0	0	0
				Conjunctivae	0	0	0	0
		Male	184-1002	Cornea	0	0	0	0
				Iris	0	0	0	0
				Conjunctivae	0	0	0	0
	Male	184-1003	Cornea	0	0	0	0	
			Iris	0	0	0	0	
			Conjunctivae	0	0	0	0	
Left eye	Control group "0.9% saline"	Male	184-1001	Cornea	0	0	0	0
				Iris	0	0	0	0
				Conjunctivae	0	0	0	0
		Male	184-1002	Cornea	0	0	0	0
				Iris	0	0	0	0
				Conjunctivae	0	0	0	0
	Male	184-1003	Cornea	0	0	0	0	
			Iris	0	0	0	0	
			Conjunctivae	0	0	0	0	



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Appendix 1-2. Individual Animal Grade in Clinical Observation of Rabbits

Applied Regions (Ocular) Non-polar group	Treated article	Gender	Animal No.	Items for Grading	Clinical Observation (time point/h)				
					1	24	48	72	
Right eye	Treatment group 0.5% Virol-Oxy extract by "cottonseed oil"	Male	184-1004	Cornea	0	0	0	0	
				Iris	0	0	0	0	
				Conjunctivae	0	0	0	0	
		Male	184-1005	Cornea	0	0	0	0	
				Iris	0	0	0	0	
				Conjunctivae	0	0	0	0	
	Male	184-1006	Cornea	0	0	0	0		
			Iris	0	0	0	0		
			Conjunctivae	0	0	0	0		
	left eye	Control group "cottonseed oil"	Male	184-1004	Cornea	0	0	0	0
					Iris	0	0	0	0
					Conjunctivae	0	0	0	0
Male			184-1005	Cornea	0	0	0	0	
				Iris	0	0	0	0	
				Conjunctivae	0	0	0	0	
Male		184-1006	Cornea	0	0	0	0		
			Iris	0	0	0	0		
			Conjunctivae	0	0	0	0		




Appendix 3. Test Article Information Sheet

表單編號：MS-A-QP03-TR004-20200605-01

Master Laboratory Co. Ltd.

Information for Test Article / Control Article

Sponsor Company	Watch Water (S) Pte Ltd
Sponsor Address	25 International Business Park #01-26/29 German Centre Singapore 609916
Contract study item	<input checked="" type="checkbox"/> Base on the contract <input type="checkbox"/> Others:
Name of test article	<u>0.5% Virol-Oxy</u>
Major components	<u>1.Potassium Pentasulfate, 2.Hydrogen Peroxide, 3.Titanium Dioxide (Crystalline Powder), 4.Sulfamin Acid, 5. Sodium Chloride</u>
Sample status	<input type="checkbox"/> Sterilized (<input type="checkbox"/> Gamma <input type="checkbox"/> EO <input type="checkbox"/> Steam) <input checked="" type="checkbox"/> Not Sterilized
Storage condition	<input checked="" type="checkbox"/> Room temperature (20°C~30°C) <input type="checkbox"/> 4°C <input type="checkbox"/> Dry <input type="checkbox"/> Away from light <input type="checkbox"/> Others:
Expiry day	3 Years Shelf-Life Time (Powder Form)
Specific requirement	
Batch/ Lot number	<input checked="" type="checkbox"/> Base on the specific number on the package: _____ <input type="checkbox"/> Base on the date on the package <input type="checkbox"/> Base on the arrived date <input type="checkbox"/> Others:
Extract by	<input checked="" type="checkbox"/> Weight (0.2g/ml) Total weight of each test article: _ <input type="checkbox"/> Surface (Sample thickness: <input type="checkbox"/> >1.0mm <input type="checkbox"/> 0.5-1.0mm <input type="checkbox"/> <0.5mm) Total area surface of each test article:
Absorption	<input checked="" type="checkbox"/> Non absorption <input type="checkbox"/> Water absorption rate: _____ / Oil absorption rate: _____
Sponsor Signature	

MS-A(C)-QP03-TR004 v2.4



Appendix 4. Test Article

