



Eleclean Disinfectant(Reactive Oxygen Species Solution) Reconstructed Human Cornea-like Epithelium (RhCE) Test for Eye Damage

FINAL REPORT

Sponsor: ELECLEAN Co., Ltd.
Testing Institution: SGS Taiwan Ltd.
Ultra Trace & Industrial Safety Hygiene
Report No.: UG/2020/51833A-01

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STUDY SCHEDULE

Eleclean Disinfectant(Reactive Oxygen Species Solution) Reconstructed Human Cornea-like Epithelium (RhCE) Test for Eye Damage

| | |
|-------------------------------|---|
| Report No.: | UG/2020/51833A-01 |
| Test Article Received Date | 2020.05.27 |
| Experimental Starting Date: | 2020.06.18 |
| Experimental Completion Date: | 2020.07.02 |
| Study Completion Date: | See Study Director's signature date in the report |
| Name of Study Personnel: | Tanya Tan |

ADDRESS INFORMATION

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Study Director

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Sponsor

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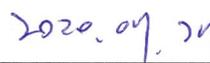
SIGNATURE OF PERSONNEL

Eleclean Disinfectant(Reactive Oxygen Species Solution) Reconstructed Human Cornea-like Epithelium (RhCE) Test for Eye Damage

Approval Signatory:



Benson Liu / SGS Taiwan Ltd.

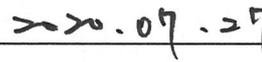


Date

Laboratory Head:



Shin Jyh Chen / SGS Taiwan Ltd.



Date

* Approval signatory of this study is the study director.

ABSTRACT

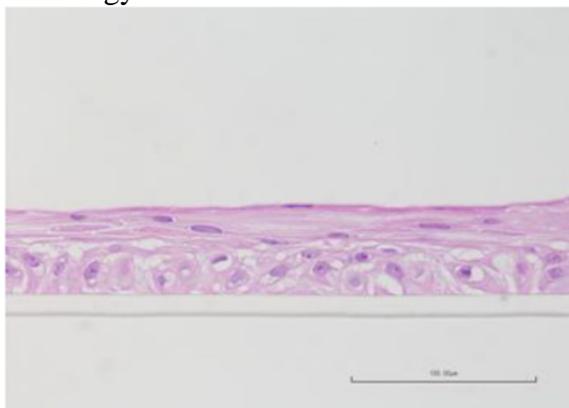
In vitro Reconstructed Human Cornea-like Epithelium (RhCE) Test for Eye Damage was performed in this study to evaluate the eye irritation or serious damage potency of “Eleclean Disinfectant(Reactive Oxygen Species Solution)”. Treatment of reconstructed human cornea-like epithelium (RhCE) tissue with test article was performed according to OECD 492 guidance. Tissue viability determined by MTT assay showed that the test article solution had in average 106.9% tissue viability. The results is considered as non-irritant or serious damage to eye in accordance with UN GHS No Category and suggested that the test article has an expected *in vivo* eye irritancy potential in the non-irritating range under this test system.

EXPERIMENTAL DESIGN

1. Test System

A. Reconstructed Human Cornea-like Epidermis: CORNEA-MODEL24, Batch No.: LCC24-200622-A, supplied by J-TEC.

B. Histology:



2. Reagents

| | Reagents | Brand | Cat No. | Lot No. |
|----|--|-----------|------------|----------------|
| A. | 10X Phosphate buffer solution (PBS) | NuAire | NU-543-600 | BSC-07 |
| B. | Ethanol | Honeywell | 34870 | J252M |
| C. | LabCyte CORNEA-MODEL 24 | J-TEC | 411124 | LCC24-200622-A |
| | Assay medium | | 402030 | 1002239874 |
| D. | 2-Propanol | MARCON | 3043-10 | 225262 |
| E. | 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) | SIGMA | M5655 | MKCG3023 |
| F. | cell counting kit-8 | SIGMA | 96992 | BCCC5910 |
| G. | EBSS | GIBCO | 14155 | 2193602 |

3. Equipments

| | Equipments | Brand | Model | Equipment No. |
|----|------------------------------|---------|------------|---------------|
| A. | Balance | OHAUS | PA214C | BAL-17 |
| B. | Biological safety cabinet | NuAire | NU-543-600 | BSC-07 |
| C. | CO ₂ Incubator | NuAire | NU-5810 | INB-16 |
| D. | Water bath | KANSIN | WB212-B2 | WAB-02 |
| E. | Microplate Spectrophotometer | BioTek™ | Eon | MPS-02 |

4. Preparation of Test Article and Controls

A. Test Article

The test article was applied directly onto the reconstructed human cornea-like epithelium. The pH adjustment; filtration and centrifugation were not conducted.

B. Controls

- (1) Positive control: 2-Hydroxyethyl acrylate.
- (2) Negative control: Ethanol

5. *In vitro* Reconstructed Human Cornea-like Epithelium (RhCE) Test for Eye Damage

- (1) Equilibrate the reconstructed human cornea-like epithelium tissue at 37±1°C, 5±1% CO₂ and ≥ 90% humidity for 24±2 hours.
- (2) After the appropriate tissue preparation, 50±2µL DPBS with test article 30±2 µL test article or controls were added to each well of RhCE tissue. Treatment on 2 tissue duplicates per condition.
- (3) After 30±2 min exposure at 37±1°C, 5±1% CO₂ and ≥ 90% humidity, each insert was individually removed from its well and rinsed with DPBS to remove any residual material.
- (4) Transfer tissues to fresh maintenance medium and incubated at 37±1°C, 5±1% CO₂ and ≥ 90% humidity for 30±2 min.
- (5) Transfer tissues to 0.3 mL/well of the warm EBSS diluted WST-8 medium for 4 h±20 min at 37±1°C, 5±1% CO₂ and ≥ 90% humidity.
- (6) After incubation, 200 uL aliquot of each well was subjected to a microplate reader equipped with a 450 nm filter for OD reading.
- (7) With the absorbance of the negative control defined as 100%, the percent absorbencies of the other article were determined. The percentages listed below directly correlate with the cell metabolism in the reconstructed human cornea-like epithelium.

6. Quality criteria

- (1) Positive and negative controls should be included in every test.
- (2) Negative control: the mean OD value of the duplicate tissues is ≤ 1.6 to ≥ 0.5 and the standard deviation (SD) of the % viability is ≤ 18%.
- (3) Positive control: the mean % viability of the PC is ≤ 40% and the SD is ≤ 18%.
- (4) Test article: the SD of % viability is ≤ 18%.

DATA MANAGEMENT

The OD values was used to calculate the percentage of viability normalised to the negative control, which is set to 100%. The quantitative data was showed as mean and standard deviation (SD). As the prediction model, the achievement of relative tissue viability is more than 40% (>40%) of the negative control is considered as non-irritant or serious damage to eye in accordance with UN GHS No Category.

| Prediction model | |
|--------------------------------------|------------------|
| Mean tissue viability is $\leq 40\%$ | No prediction |
| Mean tissue viability is $> 40\%$ | No Category (NC) |

RESULTS

OD value

| Groups | Exp 1 | Exp 2 | Exp 3 | Mean | SD |
|------------------|-------|-------|-------|-------|-------|
| Negative control | 1.286 | 1.200 | 1.094 | 1.193 | 0.096 |
| Positive control | 0.215 | 0.272 | 0.174 | 0.220 | 0.049 |
| UG/2020/51833 | 1.227 | 1.308 | 1.291 | 1.275 | 0.043 |

Tissue viability (%), normalised to the negative control

| Groups | Exp 1 | Exp 2 | Exp 3 | Mean | SD |
|------------------|-------|-------|-------|-------|-----|
| Negative control | 107.8 | 100.6 | 91.7 | 100.0 | 8.1 |
| Positive control | 18.0 | 22.8 | 14.6 | 18.5 | 4.1 |
| UG/2020/51833 | 102.8 | 109.6 | 108.2 | 106.9 | 3.6 |

CONCLUSION

Tissue viability determined by MTT assay showed that the “Eleclean Disinfectant(Reactive Oxygen Species Solution)” solution had in average 106.9% tissue viability. The results is considered as non-irritant or serious damage to eye in accordance with UN GHS No Category and suggested that the test article has an expected *in vivo* eye irritancy potential in the non-irritating range under this test system.



REFERENCES

1. Reconstructed human Cornea-like Epithelium (RhCE) test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage. TG 492 (2016).
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8. SGS SOP: EOMP-USL-0037 Manual of freezer-refrigerators. Version 1.0
9. SGS SOP: EOMP-USL-0038 Microorganism incubator operating Procedures Version 1.0
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TEST ARTICLE PHOTO

UG/2020/51833



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