



Skin Irritation Test

Study # LNH-S-095-003



FINAL REPORT

4717 Campus Drive, Kalamazoo, MI 49008

TITLE: Skin Irritation Test (OECD 439) of One Test Article

GLP STUDY: No

KEY NUMBERS

STUDY ID: LNH-S-095-003

SPONSOR ID: N/A

DATES

EXPERIMENTAL START: June 2025

EXPERIMENTAL TERMINATION: June 2025

STUDY FINALIZATION: July 2025

TESTING FACILITY: LifeNet Health LifeSciences

In Vitro Assay Services

4717 Campus Dr.

Kalamazoo, MI 49008

REQUIRED SIGNATURES

LIFENET HEALTH REPRESENTATIVE:

Signed by:
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7/8/2025

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/DATE



Table of Contents

1. Title of Study3

2. Purpose of Study.....3

3. Sponsor3

4. Testing Facility3

5. Compliance Statement3

6. Quality Assurance.....3

7. Introduction.....3

8. Test System Identification and Justification4

9. Materials and Methods.....4

9.1. Test Article 4

9.2. Materials/Reagents..... 4

9.3. Test Article Pre-Testing..... 4

9.4. Test Article Exposure 5

9.5. Tissue Viability Assay (MTT Assay) 5

9.6. Data Analysis 6

10. Results6

11. Summary and Conclusions7

12. References7

1. Title of Study

Skin Irritation Test (OECD 439) of One Test Material

2. Purpose of Study

The purpose of this study was to evaluate the dermal irritation potential for one test article, Hirudin Topical Serum (2%), in accordance with OECD Test Guideline 439 using the MatTek EpiDerm™ reconstructed human epidermis model.

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4. Testing Facility

LifeNet Health LifeSciences

4717 Campus Drive
Kalamazoo, MI 49008

5. Compliance Statement

This study was **not** conducted in compliance with Good Laboratory Practice (GLP) 21 CFR Part 58 or 40 CFR Part 160 or Part 792 but was performed according to OECD Test Guideline 439. This study also followed applicable internal SOPs.

6. Quality Assurance

This study was **not** subject to periodic inspections nor was the draft report reviewed by LifeNet Health Quality Assurance.

7. Introduction

The potential for chemicals to induce skin irritation, defined by the UN GHS as the production of reversible damage to the skin after exposure to a substance or mixture, is an important consideration when evaluating new chemicals, compounds, and topical products. Because of the role systemic reactions play in regulating local skin toxicity potential of chemicals, skin irritation potential may be predicted by in vitro systems that can sufficiently mimic the in vivo skin barrier and cell reactivity. In

this study, the MatTek EpiDerm™ Skin Irritation Test (OECD 2021; MatTek 2022) was used to evaluate the dermal irritation potential of a Sponsor-provided test article.

8. Test System Identification and Justification

The test system was the EpiDerm™ reconstructed human epidermis model from MatTek (Ashland, MA; Cat# EPI-200) which consists of normal human-derived epidermal keratinocytes with organized basal, spinous, and granular layers, as well as a multilayered stratum corneum with intercellular lamellar lipid layers with patterns analogous to those in vivo. This test system has been validated for use for in vitro assessments of skin irritation potential (OECD 2021; MatTek 2022). The EpiDerm™ tissues from MatTek are one of the acceptable skin models validated for use in accordance with OECD Test Guideline 439, and as such, has no distinct advantages over any of the other models defined in the OECD test guideline. Bias is not a factor.

9. Materials and Methods

9.1. Test Article

Name: Hirudin Topical Serum (2%)

Storage: Room Temp, protect from light

Note: this is the only information provided for this test article.

9.2. Materials/Reagents

EpiDerm™ Tissues were purchased from MatTek (Cat# EPI-200, Lot# 42124).

EpiDerm™ Assay Medium was purchased from MatTek (Cat# EPI-100-NMM, Lot# 061825TVKE).

Sterile Dulbecco's Phosphate Buffered Saline (DPBS) was purchased from MatTek (Cat# TC-PBS, Lot# 010925HDDA) and was used as a negative control.

5% Sodium Dodecyl Sulfate (SDS) was purchased from MatTek (Cat# TC-SDS 5%, Lot# 012825TVKG) and was used as a positive control.

MTT Concentrate (Cat# MTT-100-CON, Lot# 061125RAHA) and MTT Diluent (Cat# MTT-100-DIL, Lot# 050125HDDD) were purchased from MatTek to prepare the MTT solution.

Isopropanol was purchased from MatTek (Cat# MTT-100-EXT, Lot# 020725HDDA) and was used as the extractant solution.

9.3. Test Article Pre-Testing

Prior to evaluating dermal irritation, color interference of the test article was assessed. As the test article was not intrinsically colored, only one set of color interference tests was performed. 30 µL of the test article was added to 0.3 mL of deionized water in clear sealed tube, and the mixture was incubated for 1 hour at 37°C with 5% CO₂ in a humidified incubator. Then, the mixture was shaken by hand and the

presence and intensity of staining was evaluated visually. The solution did not change color substantially.

To evaluate the potential of the test article to interfere with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, 30 μ L of the test article was added to 1 mL of 1 mg/mL MTT solution in a well of a 6-well plate, and the mixture was incubated for 60 minutes at 37°C with 5% CO₂ in a humidified incubator, with untreated MTT solution used as a control. The solution did not turn blue/purple by visual inspection.

9.4. Test Article Exposure

The skin irritation test was performed per applicable internal SOPs and in accordance with OECD Test Guideline 439 and associated methods (MatTek, 2022). Upon receipt, the EpiDerm™ tissues were removed from the agarose shipping media and any agarose stuck to the outside of the tissue was removed using a sterile cotton-tipped swab (or equivalent). Tissues were placed into 6-well plates containing 0.9 mL media, taking care to ensure there were no bubbles present under the tissue. Once all tissues were transferred from the shipping agarose to the 6-well plates, the tissues were incubated for 1 hour at 37°C with 5% CO₂ in a humidified incubator. After this incubation, tissues were transferred to wells of a 6-well plate containing fresh assay media (taking care to avoid bubbles under the tissue) and incubated overnight (18 $\pm$ 3 hours) under standard culture conditions (37°C with 5% CO₂ in a humidified incubator).

The following day, 30 μ L of the test article, negative control, and positive control were applied topically to replicates of three tissues. Dosing of tissues was performed at necessary time intervals needed later for rinsing off the test and control articles (e.g. each tissue dosed 2 minutes apart). Tissues were kept in the biosafety cabinet (room temperature) until all tissues were dosed. After dosing the last tissue, plates were transferred to the humidified incubator (37°C with 5% CO₂) for 35 minutes. At the end of this incubation period, all plates were removed from the incubator and placed in the biosafety cabinet at room temperature for the completion of the 1-hour exposure. Following exposure, the tissues were rinsed thoroughly with DPBS. This was done by filling and emptying the tissue insert 15 times to remove any residual test material. The tissue was then blotted dry with a sterile cotton-tipped swab (or equivalent) to remove the DPBS. After rinsing, tissues were placed in the wells of a 6-well plate containing 0.9 mL fresh assay media, taking care to ensure there were no bubbles present under the tissue. Once all tissues were washed and placed in fresh media, the plates were transferred to a humidified incubator (37°C with 5% CO₂) for 24 hours. At the end of the 24-hour incubation period, tissues were placed in the wells of a 6-well plate containing 0.9 mL fresh assay media, taking care to ensure there were no bubbles present under the tissue. Plates containing the tissues were then transferred to a humidified incubator (37°C with 5% CO₂) for 18 hours.

9.5. Tissue Viability Assay (MTT Assay)

Following post-exposure incubation period, the tissues were transferred to 24-well plates containing 300 μ L of 1 mg/mL MTT solution, taking care to ensure there were no bubbles present under the tissue. Tissues were then incubated for 3 hours minutes at 37°C with 5% CO₂ in a humidified incubator. Tissues were blotted dry on absorbent material and added to wells of a 24-well plate containing 2.0 mL

Extractant Solution (isopropanol). The plates were then covered with parafilm to prevent evaporation and gently shaken (~120 rpm) on an orbital shaker for at least two hours at room temperature. Alternatively, overnight extraction could be performed by placing the 24-well plates at room temperature in the dark, without shaking.

The tissues were then removed from the wells, and the tissue was pierced, allowing the extractant to remain in the well while the tissue was removed. The extraction solution was mixed well by pipet, and 200 µL aliquots of the extract solutions was transferred to the appropriate wells of 96-well plates.

Optical Density (OD) of the extractant was read at 570 nm using a BioTek Synergy H1 Plate Reader (Agilent) or equivalent following applicable internal SOPs.

9.6. Data Analysis

Data were compiled in Excel, and the mean, standard deviation, and standard error of the mean were calculated using Excel. Graph were created as applicable using GraphPad Prism 10.

Tissue viability was calculated using the following equation:

$$\% \text{ Viability} = 100 \times [OD(\text{treated tissue}) / \text{mean } OD(\text{negative control})]$$

The test article classification was determined based on Table 9.1 below.

Table 9.1. Test Article Classification Prediction Model

In Vitro Result	In Vivo Prediction
	OECD Test Guideline 439
Mean Tissue Viability $\leq$ 50%	Requiring Classification and Labeling (GHS Category 1 or 2)
Mean Tissue Viability $>$ 50%	Non-Irritant to Skin (GHS No Category)

The assay was considered meeting the acceptance criteria if the mean OD of the negative control tissues was $\geq$ 1.0 and $\leq$ 2.8, the mean viability of the positive control tissues was $<$ 20% relative to the negative control, and the SD calculated from individual % tissue viabilities of the 3 replicates was $\leq$ 18%.

10. Results

The assay met the acceptance criteria as the mean background subtracted OD for the negative control tissues was 2.082 with individual tissue ODs of 2.168, 1.877, and 2.201. The mean viability of the positive control was 2.5% relative to the negative control. The SD calculated from individual % tissue viabilities of the 3 replicates was 8.6% for the negative control, 0.2% for the positive control, and 4.5% for the test article.

The mean viability for the test article exposed tissues was 96.8% viable relative to the negative control.

Table 10.1. Test Article Classification Prediction Model

Sponsor's Designation	Lot/Batch #	Conc.	Mean Viability (%) $\pm$ SD	Skin Irritation Prediction	pH
Negative Control (DPBS)	010925HDDA	Neat	100.0 $\pm$ 8.6	No Category (Non-Irritant)	ND
Positive Control (5% SDS)	012825TVKG	5% (w/v)	2.5 $\pm$ 0.2	Requiring Classification and Labeling (Category 1 or 2)	ND
Hirudin Topical Serum (2%)	NP	Neat	98.6 $\pm$ 4.5	No Category (Non-Irritant)	ND

ND = not determined

NP = not provided

11. Summary and Conclusions

The assay fit all acceptance criteria and was therefore deemed a valid run. The mean viability of tissues exposed to the test articles was 98.6% viable compared to negative control, resulting in a classification of No Category (Non-Irritant).

12. References

OECD (2021), Test No. 439: In Vitro Skin Irritation: Reconstructed Human Epidermis Test Method, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, <https://doi.org/10.1787/9789264242845-en>.

MatTek (2022), In Vitro EpiDerm™ Skin Irritation Test (EPI-200-SIT) For Use with MatTek Corporation's Reconstructed Human Epidermal Model EpiDerm™ (EPI-200-SIT).

NOTE: In the event the sponsor does not provide comments within 30 days of receipt of the draft report, the report will be finalized and signed.