iuvo bioscience
Partner Research Organization

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Sponsor:
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iuvo Test No.: T20-1431

iuvo Study No.: GLP-2020-0120

Final Report

AGAR DIFFUSION TEST
QUALITATIVE CYTOTOXICITY ASSESSMENT (ISO METHOD)

of

iBrea Skin Relief Intense Recovery Cream
Lot Identification: N/A

Written and
Approved By:

Annetta Herrington / Principal Scientist, Toxicology
Study Director

07-16-2020

Study Completion Date

Test Facility: iuvo BioScience (iuvo)
7500 West Henrietta Road
Rush, New York 14543

This laboratory is accredited in accordance with the recognized International Standard ISO/IEC 17025:2017. This accreditation demonstrates technical competence for a defined scope and the operation of a laboratory quality management system. This test falls within the scope of accreditation.

Test Received: 06/16/2020

Study Initiation Date: 06/23/2020

Test Performance Dates: 06/24/2020 – 06/25/2020

Test Articles: iuvo has received and evaluated the following samples:

iBrea Skin Relief Intense Recovery Cream
Lot Identification: N/A

Storage conditions at test facility: room temperature.

Stability testing by sponsor: in progress.

Test article description: thick white cream

Intended use: cosmetics

Control Articles: Positive Control: Sterile Latex Rubber (Mfr. / Lot: Indonesia / 812407HT).

Negative Control: Sterile USP High Density Polyethylene Reference Standard (Lot: K0M357).

Filter Paper Control: Sterile filter paper (Mfr. / Lot: Whatman / 9816838) saturated with USP Water for Irrigation (WFI Mfr. / Lot / Exp.: B/Braun / J9L958 / 09-30-2022)

The control articles were stable under the conditions of the assay, have been validated for use in the test system, and have consistently provided suitable results.

Purpose of The Study:

The Agar Diffusion Test is an *in vitro* procedure by indirect contact which allows for a qualitative assessment of cytotoxicity. This test is not appropriate for leachables that cannot diffuse through the agar layer, or that may react with agar. The use of this assay for the assessment of cytotoxicity to meet ISO 10993-5:2009(E) requirements shall be justified. The United States Pharmacopeia recommends this test for the evaluation of elastomeric closures in a variety of shapes.

Test System And Justification:

The L929 mammalian fibroblast was chosen as the test system for this study because it is a sensitive, dependable cell line which has historically been used to evaluate biomaterials for cytotoxic effects. The L929 mammalian fibroblast is an established cell line obtained from a recognized repository (American Tissue Culture Collection CCL 1, NCTC clone 929). The International Standard, ISO 10993-5:2009(E), endorses the suitable use of this cell line.

Methods:

L929 cells were grown and maintained in Complete MEM [Minimum Essential Medium (Eagles) with Earles Balanced Salts supplemented to contain 10% fetal bovine serum (FBS) and an antibiotic-antimycotic solution]. For this test, confluent stock cultures were trypsinized and replated in 60 mm sterile, tissue culture grade petri plates at a density of approximately 1 x 10^6 cells/plate. All plates were incubated for not less than twenty-four (24) hours at 37 ± 1°C in a humidified atmosphere containing 5 ± 1% CO_2 in air to obtain approximately 80% confluent monolayers for testing.

Following incubation, the subconfluency and morphology of the cultures were verified using a microscope. Cultures containing approximately 80% confluency and appearing as normal, healthy cells were used. The medium was carefully removed from each monolayer and replaced with 7 ml of a 1:1 mixture of 2% w/v purified agar and 2X Complete MEM. The agar overlay was allowed to solidify at room temperature for at least 15 minutes. Prior to testing, the cell monolayers were stained with 2-5 ml of a 0.01% neutral red vital stain solution. The stain remained on the plates for a minimum of fifteen (15) minutes and was then carefully removed by aspiration.

The test article was evaluated as received (neat). A volume of 0.2 ml of test material was applied to sterile filter paper and plated test material side down to cover approximately 10% of the cell agar layer surface (~2.1 cm^2). A filter paper blank saturated with 0.2 ml WFI was prepared in likewise fashion to mimic the test article application. Standard positive and negative experimental controls were prepared by aseptically cutting 2.1 cm^2 pieces of latex rubber and USP High Density Polyethylene Reference Standard and placing them onto the agar surface of additional plates. All test and experimental control plates were prepared in triplicate. Three (3) plates were also prepared to serve as blank controls. All plates were incubated for twenty-four (24) to twenty-eight (28) hours at 37 ± 1°C in a humidified atmosphere containing 5 ± 1% CO_2 in air.

Cytotoxic Evaluation:

Following incubation, all plates were microscopically scored for qualitative biological reactivity (cellular degeneration and malformation) as follows:

Grade	Reactivity	Description of Reactivity Zone
0	None	No detectable zone around or under specimen
1	Slight	Some malformed or degenerated cells under specimen
2	Mild	Zone limited to area under specimen
3	Moderate	Zone extending specimen size up to 1.0 cm
4	Severe	Zone extending greater than 1.0 cm beyond specimen

Test Criteria:

The cell culture test system is suitable if the observed responses to the negative control is grade 0 (no reactivity) and to the positive control is at least grade 3 (moderate reactivity). The test article meets the requirements of the test if the response to the test article is not greater than grade 2 (mildly reactive). The achievement of a grade greater than 2 is considered a cytotoxic effect. The test must be repeated if the negative, positive and any other controls do not have the expected response in the test system. If there are evident differences in the test result for replicate culture vessels, then the test is either inappropriate or invalid for the evaluation of the test sample and the test shall be repeated or an alternative methodology used.

Results:

The test material, iBrea Skin Relief Intense Recovery Cream, Lot Identification: N/A, was tested via Agar Diffusion Test following the FDA guideline for GLP testing.

Results are presented in Table I of this report. Suitability of the test system was confirmed. The test results were consistent among all replicates.

There were no circumstances during the course of this study that negatively affected the quality or integrity of the data obtained.

Summary/ Conclusions:

The test article, iBrea Skin Relief Intense Recovery Cream, Lot Identification: N/A, induced mild reactivity (grade 2). Since the reactivity was not greater than grade 2 (mild), the test article is not considered to elicit a cytotoxic effect under the conditions employed.

Records:

All raw data, documentation, protocols and final reports generated for this study are retained for a minimum of five (5) years in the archives by iuvo BioScience, 7500 West Henrietta Road, Rush, New York 14543. After five (5) years, the sponsor will be contacted to determine disposition of documentation.

Quality Assurance: The Quality Assurance Unit conducted inspections of the protocol, during incubation period, and reviewed the final report.

A signed statement prepared by the Quality Assurance Unit is included with this final report. This study was conducted in compliance with the Good Laboratory Practice Regulations of Title 21, CFR: Part 58.

References:

(1) ISO 10993-5:2009(E), Biological Evaluation of Medical Devices - Part 5: Tests for *In Vitro* Cytotoxicity.

(2) United States Pharmacopeia and National Formulary, <87> Biological Reactivity Tests, *In Vitro*, Current Edition.

(3) iuvo Procedure CYT-2: Agar Diffusion Test (USP and ISO)

Study Personnel:

Annetta Herrington	Principal Scientist, Toxicology / Study Director
Kayla Feeney	Technician II, Toxicology

Results

AGAR DIFFUSION TEST
QUALITATIVE CYTOTOXICITY ASSESSMENT (ISO METHOD)
of

Test Article: iBrea Skin Relief Intense Recovery Cream
Lot Identification: N/A

Sample Description	Grade (plates 1, 2, 3)	Reactivity
Test Article	2, 2, 2	Mild
Positive Control	3, 3, 3	Moderate
Negative Control	0, 0, 0	None
Filter Paper/Vehicle Control	0, 0, 0	None
Blank	Normal, Healthy Cells	

End of Report

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STATEMENT OF GLP QUALITY ASSURANCE ACTIVITIES

FOR NONCLINICAL LABORATORY STUDIES
Reference REG-3, REG-4, FDA 21 CFR 58

Study Information

Study Number: GLP-2020-0120
Test Number(s): T20-1431
Study Title: Agar Diffusion Test Qualitative Cytotoxicity Assessment (ISO Method)
Sponsor: IBREA GLOBAL CO., LTD

Phase Inspected	Date Inspected	iuvo Management / Principal Investigator Notification Date	Study Director Notification Date
Protocol Review	24 June 2020	24 June 2020	24 June 2020
During Incubation Period	24 June 2020	24 June 2020	24 June 2020
Study Data and Draft Report Review	15 July 2020	16 July 2020	16 July 2020
Final Report Review	16 July 2020	16 July 2020	16 July 2020

Based on review of this nonclinical laboratory study, it has been concluded that this report accurately describes applicable methods and procedures and that the reported results accurately reflect the raw data. This study was reviewed in accordance with the provisions of the FDA Good Laboratory Practice Regulations (21 CFR, Part 58).

iuvo Quality Assurance: Joanna Cyr Date: 16 July 2020