Nelson Labs
A Sotera Health company

Sponsor:
Melanie Choi
Savewo Limited
Flat B1, 10/F, Block B, Yee Lim Industrial Centre
No 2-28 Kwai Lok Street
Kwai Chung, New Territories
Hong Kong,
HONG KONG

MEM Elution Final Report

Test Article:	SAVEWO 3DMASK
Study Number:	1330326-S01
Study Received Date:	12 Aug 2020
Testing Facility:	Nelson Laboratories, LLC 6280 S. Redwood Rd. Salt Lake City, UT 84123 U.S.A.
Test Procedure(s):	Standard Test Protocol (STP) Number: STP0032 Rev 10
Deviation(s):	None

Summary: The Minimal Essential Media (MEM) Elution test was designed to determine the cytotoxicity of extractable substances. An extract of the test article was added to cell monolayers and incubated. The cell monolayers were examined and scored based on the degree of cellular destruction. All test method acceptance criteria were met. Testing was performed in compliance with US FDA good manufacturing practice (GMP) regulations 21 CFR Parts 210, 211 and 820.

Results:

Test Article:

Dilution	Results Pass/Fail	Scores				Extraction Ratio	Amount Tested / Extraction Solvent Amount
		#1	#2	#3	Average		
Neat	**Pass**	2	2	2	2	3 cm^2/mL	534.1 cm^2 / 178 mL
1:2	**Pass**	1	1	1	1		
1:4	**Pass**	1	0	1	1		
1:8	**Pass**	0	0	0	0		
1:16	**Pass**	0	0	0	0		

Note: An additional 12 mL of media was added to account for absorbency.

Danielle Short electronically approved — 25 Aug 2020 20:12 (+00:00)
Study Director Danielle Short — Study Completion Date and Time

Controls:

Identification	Scores				Extraction Ratio	Amount Tested / Extraction Solvent Amount
	#1	#2	#3	Average		
Negative Control - Polypropylene Pellets	0	0	0	0	0.2 g/mL	4 g / 20 mL
Media Control	0	0	0	0	N/A	20 mL
Positive Control - Latex Natural Rubber	4	4	4	4	0.2 g/mL	4 g / 20 mL

Test Method Acceptance Criteria: The United States Pharmacopeia & National Formulary (USP <87>) states that the test article meets the requirements, or receives a passing score (**Pass**) if the reactivity grade is not greater than grade 2 or a mild reactivity. The ANSI/AAMI/ISO 10993-5 standard states that the achievement of a numerical grade greater than 2 is considered a cytotoxic effect, or a failing score (**Fail**).

Nelson Laboratories acceptance criteria was based upon the negative and media controls receiving "0" reactivity grades and positive controls receiving a 3-4 reactivity grades (moderate to severe). The test was considered valid as the control results were within acceptable parameters.

The cell monolayers were examined microscopically. The wells were scored as to the degree of discernable morphological cytotoxicity on a relative scale of 0 to 4:

Conditions of All Cultures	Reactivity	Grade
No cell lysis, intracytoplasmic granules.	None	0
Less than or equal to 20% rounding, occasional lysed cells.	Slight	1
Greater than 20% to less than or equal to 50% rounding, no extensive cell lysis.	Mild	2
Greater than 50% to less than 70% rounding and lysed cells.	Moderate	3
Nearly complete destruction of the cell layers.	Severe	4

The results from the three wells were averaged to give a final cytotoxicity score.

Procedure: The amount of test material extracted was based on ANSI/AAMI/ISO and USP surface area or weight recommendations. Test articles and controls were extracted in 1X Minimal Essential Media with 5% bovine serum for 24-25 hours at 37 ± 1°C with agitation. Multiple well cell culture plates were seeded with a verified quantity of industry standard L-929 cells (ATCC CCL-1) and incubated until approximately 80% confluent. The test extracts were held at room temperature for less than four hours before testing. The extract fluids were not filtered, centrifuged or manipulated in any way following the extraction process. The test extracts were added to the cell monolayers in triplicate. The cells were incubated at 37 ± 1°C with 5 ± 1% CO_2 for 48 ± 3 hours.

Pre and Post Extract Appearance		
Test Article	Pre extract	Clear with no particulates present
	Post extract	Clear with no particulates present No color change noted
Controls	Pre extract	Clear with no particulates present
	Post extract	Clear with no particulates present No color change noted

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中国认可
国际互认
检测
TESTING
CNAS L2954

Final Report

Report Number: SDWH-M202106929-1 (E)

In Vitro Cytotoxicity Test of Savewo ClassicMask

According to ISO 10993-5: 2009
MTT Method
MEM with 10%FBS extract

Sponsor: Savewo Limited

Address: 1/F, 266-270 Texaco Road, Tsuen Wan, Hong Kong

Sanitation & Environment Technology Institute, Soochow University
Address: 199 Ren-Ai Road, Suzhou Industrial Park, Suzhou, Jiangsu 215123, P. R. China
Website: www.sudatest.com E-mail: med@sudatest.com
Direct: +86 512 65880038 Free: 400 107 8828

Content

Supplementary Explanation

(1) Please apply for rechecking within 15 days of receiving the report if there are any objections.

(2) Any erasure or without special inspection and testing seal renders the report null and void.

(3) The report is only valid when signed by the persons who edited, checked and approved it.

(4) The results relate only to the articles tested.

(5) The report shall not be reproduced except in full without the written approval of the institute.

Quality Assurance Statement

The Quality Assurance Unit inspected/audited this study in compliance with the following GLP regulations:

Good Laboratory Practice (GLP) Regulation 21 CFR Part 58, U.S. Food and Drug Administration (FDA).

The Quality Assurance Unit conducted inspections on the following dates. The findings were reported to the Study Director and to the Testing Facility Management. The final report was reviewed by the Quality Assurance Unit. The final report accurately describes the test methods in accordance with standard operating procedures, and the results are consistent with raw data of non-clinical studies conducted according to the study protocol.

Inspections	Date of Inspection	Date Reported to Study Director	Date Reported to Testing Facility Management.
Study Protocol	2021-12-17	2021-12-17	2021-12-28
Study Procedure	2021-12-23	2021-12-23	2021-12-28
Raw Data	2021-12-28	2021-12-28	2021-12-28
Final Report	2021-12-28	2021-12-28	2021-12-28

Quality Assurance Unit: Ou Tingting 2021-12-28

Quality Assurance Date

GLP Compliance Statement

This study was fully in accordance with the technical requirements of the study protocol.

This study was conducted in compliance with Good Laboratory Practice (GLP) Regulation 21 CFR Part 58, U.S. Food and Drug Administration (FDA).

Verification Dates

Test Article Receipt	2021-12-15
Protocol Effective Date	2021-12-17
Technical Initiation Date	2021-12-19
Technical Completion Date	2021-12-24
Final Report Completion Date	2021-12-28

Edited by: Xia yousu 2021-12-28
Date

Reviewed by: Zhu Yuting 2021-12-28
Study Director Date

Approved by: Wang Yijie 2021-12-28
Authorized Signatory Date

Sanitation & Environment Technology Institute, Soochow University

Summary

1 Test Article

Test Article Name	Savewo ClassicMask
Manufacturer	Savewo Limited
Address	1/F, 266-270 Texaco Road, Tsuen Wan, Hong Kong
Model	ClassicMask
Lot/Batch	Lot 21212280

2 Main Reference

ISO 10993-5: 2009 Biological evaluation of Medical Devices—Part 5: Tests for *in vitro* Cytotoxicity

3 Test Method

Potential toxicity of test article was evaluated using MTT in accordance with ISO 10993-5: 2009 Biological evaluation of Medical Devices—Part 5: Tests for *in vitro* Cytotoxicity.
Study protocol number: SDWH-PROTOCOL-GLP-M202106929-1 .

4 Conclusion

Under the conditions of this study, the test article extract showed potential toxicity to L929 cells.

Test Report

1 Purpose

The purpose of the test is to determine the biological reactivity of a mammalian cell culture (mouse fibroblast L929 cells) in response to the test article.

2 Reference

ISO 10993-5: 2009 Biological evaluation of Medical Devices— Part 5: Tests for *in vitro* Cytotoxicity

3 Compliance

Good Laboratory Practice Regulations, 21 CFR, Part 58.
ISO/IEC 17025: 2017 General requirements for the competence of testing and calibration laboratories (CNAS—CL01 Accreditation criteria for the competence of testing and calibration laboratories) China National Accreditation Service for Conformity Assessment LABORATORY ACCREDITATION CERTIFICATE Registration No. CNAS L2954.
RB/T 214—2017 Competence assessment for inspection body and laboratory mandatory approval—General requirements for inspection body and laboratory Certification and Accreditation Administration of the People's Republic of China INSPECTION BODY AND LABORATORY MANDATORY APPROVAL Certificate No. CMA 180015144061.

4 Identification of Test and Control Articles

4.1 Test Article

Test Article Name	Savewo ClassicMask
Manufacturer	Savewo Limited
Address	1/F, 266-270 Texaco Road, Tsuen Wan, Hong Kong
Test Article Initial State	Non-sterile
CAS Number	Not supplied by sponsor (N/S)
Model	ClassicMask
Size	175 x 95mm
Lot/Batch	Lot 21212280
Raw Material	N/S
Packaging Material	N/S
Physical State	Solid
Color	White
Density	N/S
Stability	N/S
Solubility	N/S
Storage Condition	Room temperature
Intended Use	Surgical Mask is intended to be worn by operating room personnel and other general healthcare workers to protect both patients and healthcare workers against transfer of microorganism, blood, body fluids, and particulate materials.Surgical Mask is intended for use in infection control practices to reduce potential exposure to blood and body fluids.
Additional Information	Lot 21212280

The information about the test article was supplied by the sponsor wherever applicable.
The Sponsor is responsible for all test article characterization data as specified in the GLP

regulations.

4.2 Control Article

4.2.1 Negative Control

Negative Control Article Name: High Density Polyethylene

Manufacturer: U.S. Pharmacopeial Convention (USP)

Size: 3 Strips

Lot/ Batch#: R149K0

Physical State: Solid

Color: White

Stability: Stable at room temperature

Storage Conditions: Room temperature

Extraction vehicle: MEM medium, with addition 10% FBS

4.2.2 Positive Control

Positive Control Article Name: Zinc diethyldithiocarbamate

Manufacturer: Sigma

Size: 25g

Lot/ Batch#: MKCB2943V

Concentration: 1%

Solvent: MEM medium, with addition 10% FBS

Physical State: Powder

Color: White

4.2.3Blank Control

Blank Control Article Name: MEM medium, with addition 10% FBS

Physical State: Liquid

Color: Pink

Storage Condition: 4 ± 2°C

5 Equipment and Reagents

5.1 Equipment

Equipment Name	Equipment Number	Calibration Expire
Autoclave	SDWH2204	2022-03-09
Constant temperature vibrator	SDWH2109	2022-08-10
Steel straight scale	SDWH463	2022-06-29
Electronic Balance	SDWH2601	2022-05-11
Electronic Balance	SDWH3015	2022-11-21
CO_2 Incubator	SDWH2839	2022-03-09
Inverted microscope	SDWH2882	2022-06-15
Clean bench	SDWH454	2022-04-18
Power Wave Microplate Reader	SDWH2386	2022-05-13

5.2 Reagents

Reagent Name	Manufacturer	LOT
(3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyletrazolium	SIGMA	MKCL9866

bromide)		
FBS	CORNING	26219002
MEM	HyClone	AG29697698
Trypsin	GiBco	2186962
Penicillin, Streptomycin sulfate	GiBco	2289327
PBS	HyClone	AF29561133
99.9%Isopropanol	Sinopharm Chemical Reagent Co., Ltd	20210507

6 Identification of Test System

L929 mouse fibroblast cells obtained from ATCC (American Type Culture Collection), USA.

7 Justification of Test System and Route of Administration

Historically, mouse fibroblast L929 cells have been used for cytotoxicity studies because they demonstrate sensitivity to extractable cytotoxic articles.

The test article was extracted and administered in vitro to mouse fibroblast L929 cells through a solvent compatible with the test system. This was the optimal route of administration available in this test system as recommended in the guidelines.

8 Experimental Design

8.1 Preparation of Extracts

8.1.1 Pretreatment

8.1.1.1 Test samples

No additional sterilization procedures required for samples.

8.1.1.2 Control samples

Autoclaving at 121°C for 30 min.

8.1.2 Extraction

Under aseptic conditions, samples were taken according to the sampling method (Whole sampling, excluding the nose clip, add additional volume of extraction vehicle that the test sample absorbs when performing the extraction, using the data of the combined area of all tissue contacting surfaces of each sample provided by the sponsor as the standard surface area, 166.25 cm^2). Extractions shall be performed with agitation in closed inert containers according to the extraction ratio listed in the following table (sample: extraction vehicle). The extraction vehicle is MEM medium containing 10% fetal bovine serum. After the extraction was completed, record the condition of the extracts and any changes in the extraction solvent (pre- and post-extraction). The extracts will be used immediately for test.

Samples	Actual Sampling	Extract Procedure			Final Extract
		Extract Ratio	Volume of Extraction Vehicle	Condition	
Test	166.25 cm^2	3 cm^2: 1 mL	64.9 mL	37°C, 72 h	Clear
Negative Control	60 cm^2	3 cm^2: 1 mL	20.0 mL	37°C, 72 h	Clear
Blank Control	/	/	10.0 mL	37°C, 72 h	Clear
Positive Control	0.5 g	1.0 g:100 mL	50.0 mL	37°C, 72 h	Not Clear

There was no change in the extraction solvent for the test samples (pre- and post-extraction). The final extract of the test samples was not subjected to processes such as pH adjustment, filtration, centrifugation, or dilution. Only the positive control extract was filtered before use since the powder of the positive sample suspended in the extraction solvent can adversely affect the test system.

8.2 Experimental Procedure

Aseptic procedures were used for handling cell cultures.

L929 cells were cultured in MEM medium (10% FBS, Penicillin 100 U/mL, Streptomycin sulfate 100 μg/mL) at 37°C in a humidified atmosphere of 5% CO_2, then digested by 0.25% trypsin containing EDTA to get single cell suspension. And obtain a 1×10^5 cells/mL suspension by centrifuging (200 G,3 min) and re-dispersing in MEM medium finally.

The suspended cells were dispensed at 100μL per well in 96-well plate, and culture it in cell incubator (5% CO_2,37°C,>90%humidity) for 24h. Cell morphology was evaluated to verify that the monolayer was satisfactory.

After the cells grew to form a monolayer, original culture medium was discarded. The 96-well plates were then treated with 100μL of extract of test article (100%, 50%, 25%, 12.5%), control article, negative article (100%) and positive article (100%) respectively. Incubate the 96-well plate at 37°C in cell incubator of 5% CO_2 for 24 h. Five replicates of each test were tested.

After 24 h incubation, observe the cell morphology first and then discard the culture medium. A 50μL aliquot of MTT (1 mg/mL) was added to each well and then incubated at 37°C in a humidified atmosphere of 5% CO_2 for 2 h. The liquid in each well was tipped out and 100 μL 99.9% isopropanol was added to each well to suspend the cell layer.

Evaluate the suspension above with a dual-wavelength spectrophotometer with the measurement wavelength at 570 nm and reference wavelength at 650 nm.

8.3 Results

The cell viability of 100% test article extract was 61.9%. See Annex 1, table 1 and table 2 for specific results.

8.4 Quality Check

No cytotoxic effect is observed for the negative controls and a cytotoxic effect is elicited by the positive controls.

The absolute value of optical density, OD_{570}, obtained in the untreated blank indicates the 1×10^4 cells seeded per well have grown exponentially with normal doubling time during the two days of the assay.

The mean OD_{570} of blanks is not less than 0.2.

Check for systematic cell seeding errors, blanks are placed both at the left side (row 2) and the right side (row 11) of the 96-well plate (row 1 and row 12 shall not be used). The left and the right mean of the blanks do not differ by more than 15 % from the mean of all blanks.

8.5 Statistical Method

SPSS16.0 will be used to calculate the Mean ±SD of each group.

$$\text{Viab.}(\%) = 100 \times \overline{(OD_{570} - OD_{650})}_{\text{Sample}} \Big/ \overline{(OD_{570} - OD_{650})}_{\text{Blank}}$$

The lower the Viab.% value, the higher the cytotoxic potential of the test article is.

8.6 Evaluation Criteria

The lower the Viab.% value, the higher the cytotoxic potential of the test article is.
If viability is reduced to < 70 % of the blank, it has a cytotoxic potential.
The Viab.% of the 100% extract of the test article is the final result.

9 Conclusion

Under the conditions of this study, the test article extract show no potential toxicity to L929 cells.

10 Record Storage

All raw data pertaining to this study and a copy of the final report are to be retained in designated SDWH archive.

11 Confidentiality Agreement

Statements of confidentiality were as agreed upon prior to study initiation.

12 Deviation Statement

There were no deviations from the approved study protocol which were judged to have any impact on the validity of the data.

Annex 1 Results

Table 1 Observation of the Cell morphology

Group	After inoculation	Before treated with extract	24 h after treatment
Blank control	Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth.	Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth.	Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth.
Negative control			Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth.
Positive control			Nearly complete or complete destruction of the cell layers.
100% Test article extract			Occasional cells were round and with intracytoplasmatic granules, or showed changes in morphology; occasional lysed cells were present; only slight growth inhibition observable.
50%Test article extract			Occasional cells were round and with intracytoplasmatic granules, or showed changes in morphology; occasional lysed cells were present; only slight growth inhibition observable.
25% Test article extract			Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth.
12.5% Test article extract			Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth.

Table2 Results of the Cell Viability

Group	Value of OD Mean±SD	Cell Viability%
Blank control	0.9560±0.109	100.0
Negative control	0.9616±0.059	100.6
Positive control	0.1476±0.007	15.4
100% Test article extract	0.7922± 0.036	81.9
50%Test article extract	0.8298± 0.031	86.3
25% Test article extract	0.8536±0.089	89.3
12.5% Test article extract	0.9038±0.053	94.5

Annex 2 Photograph of Test Article

Annex 3 Information Provided by Sponsor

1 Production Process

Not supplied by sponsor.

2 Other Information

Not supplied by sponsor.

End of Report