FINAL REPORT

Test for *in vitro* cytotoxicity: Elution Method of Taglus Standard Thermoforming Foils as per ISO 10993 5:2009(E).

STUDY CONTRACT PARTNER:
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TEST FACILITY:
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Study No.: 073/433

STUDY SPONSOR AND APPLICANT:
Vedia Solutions
Division of Laxmi Dental Export Pvt Ltd
103, Akruti Arcade, J P Road
Opp A H Wadia School, Andheri West
Mumbai 400053

REPORT ISSUED DATE: 30 December 2021

FINAL REPORT

PRODUCT NAME:

Taglus Standard Thermoforming Foils

STUDY TITLE

Test for *in vitro* cytotoxicity: Elution Method of Taglus Standard Thermoforming Foils as per ISO 10993-5:2009(E)

STUDY NUMBER:
073/433

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444, Gokulam Street
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STUDY DIRECTOR AUTHENTICATION STATEMENT

Study No : **073/433**

Study Title : **Test for *in vitro* cytotoxicity: Elution Method of Taglus Standard Thermoforming Foils as per ISO 10993-5:2009(E)**

This study was performed in accordance with the mutually agreed study plan and GLR Laboratories Private Limited's standard operating procedures, unless otherwise stated, and the study objective was achieved. I accept overall responsibility for the technical conduct of the study, as well as for the interpretation, analysis, documentation and reporting of results. This report provides a true and accurate record of the results obtained.

This study was performed in compliance with OECD Principles of Good Laboratory Practice* ENV/MC/CHEM (98)17 (Revised 1997, issued January 1998) and applicable regulatory requirements including the US Food and Drug Administration's GLP regulations, 21 CFR 58 (subparts B to G and J).

V. Raja

30 Dec 2021

Mr. V. Rajasekar, MTech (Biotech)
Study Director
GLR Laboratories Private Limited

Study Completion Date

* The identity and composition of the test item are the responsibilities of the sponsor.

QUALITY ASSURANCE STATEMENT

Study No : **073/433**

Study Title : **Test for *in vitro* cytotoxicity: Elution Method of Taglus Standard Thermoforming Foils as per ISO 10993-5:2009(E)**

The Quality Assurance (QA) of GLR Laboratories Private Limited verified the Study Plan, including any amendments, inspected the critical study phases, audited the raw data, and report of this Study as per in-house Standard Operating Procedures (SOPs) for compliance with the OECD Principles of Good Laboratory Practice (as revised in 1997) [ENV/MC/CHEM(98)17], and for compliance with relevant regulatory requirements.

During the Study, the following study-related inspections/audits were performed on the following dates and reported to the Study Director and Test Facility Management. Besides the below, process and facility inspections were also carried out periodically at this Test Facility by auditor(s) of the QA, as per in-house SOPs, which may have relevance to this study.

S. No.	Type(s) of Study Inspection/Audit	Date(s) of Inspection/Audit	Phase(s) of Study inspected/audited	Date(s) of Reporting to Management and Study Director (Inspection No.)
1	Study Plan Verification	19 November 2021	Draft Study Plan	19 November 2021 (SBI/073/433/001)
2	Study Plan Verification	26 November 2021	Definitive Study Plan	26 November 2021 (SBI/073/433/002)
3	In Life Phase Inspection	07 December 2021	Addition of Test Item to Cell Lines	07 December 2021 (SBI/073/433/003)
4	In Life Phase Inspection	08 December 2021	Quantitative Evaluation	08 December 2021 (SBI/073/433/004)
5	Report Audit	17 December 2021	Draft Report	17 December 2021 (SBI/073/433/005)
6	Report Audit	30 December 2021	Final Report	30 December 2021 (SBI/073/433/006)

The QA has determined that the methods, procedures, observations, and reported results are accurately and completely described and that the reported results are based on the Study Plan and the pertinent raw data generated during the course of the Study. The Study Director's GLP compliance statement is supported.

N. Parthiban

30 DEC 2021

Dr. Parthiban Natarajan, PhD, ERT
Head-Quality Assurance
Asst. Director, GLR Laboratories Private Limited

Date

TEST FACILITY MANAGEMENT STATEMENT

Study No : **073/433**

Study Title : **Test for *in vitro* cytotoxicity: Elution Method of Taglus Standard Thermoforming Foils as per ISO 10993-5:2009(E)**

This is to certify that, the Test Facility Management appointed the Study Director and provided all necessary facilities and resources for the proper conduct of this study, in compliance with the Principles of OECD Good Laboratory Practice (GLP), as per the recommendations of the OECD (Council Act [C (97) 186 (Final)]) and as adopted in the procedures promulgated by the National GLP Compliance Monitoring Authority, Government of India.

Ms. M. Yaminy, B. Com, (MBA)
Deputy Test Facility Management
GLR Laboratories Private Limited

30 Dec 2021
Date

SUMMARY

The test item, Taglus Standard Thermoforming Foils supplied by Vedia Solutions, was evaluated for its ability to induce cytotoxicity in Balb/c 3T3 cells using elution method.

The test item, Taglus Standard Thermoforming Foils is a surface device which comes in contact with mucosal membrane. The dimension of the test item are:- diameter: 12.5 cm and thickness: 0.8 mm. The duration of contact is less than 24 hours (limited).

The test item was extracted at a ratio of 3 cm^2/mL (as the thickness of the test item was greater than 0.5 mm) in 1x DMEM supplemented with 5% heat inactivated new born calf serum, 4 mM L-glutamine and 1% penicillin/streptomycin solution (extraction medium) at 37 ± 1 °C for 24 h. Test item measuring 245 cm^2 (as calculated in our laboratory) was extracted in 81.7 mL of extraction medium at 37 ± 1 °C for 24 h and 20 min, under aseptic condition. Sterilized High - Density Polyethylene film (negative control) measuring 18 cm^2 (both sides) was extracted (at the ratio of 3 cm^2 per mL of solvent) in 6 mL of extraction medium at 37 ± 1 °C for 24 h and 20 min. Positive control, sodium lauryl sulphate (SLS) was freshly prepared (before treatment to the cells) at a final concentration of 0.15 mg/mL. This fulfils the requirement of ISO 10993-12:2012(E), ISO 10993-12:2021(E) and ISO 10993 5:2009(E).

At the end of extraction, the extract was clear, no colour change or particulates were observed. No changes were observed in the retrieved test item. No additional processing such as filtration, centrifugation, pH adjustments or any other processing were made. Extracts were used within 13 min and was considered stable during this time.

Exponentially growing Balb/c 3T3 cells were seeded in a 96-well plate at a concentration of 1 x 10^4 cells/well. After 24 hours of incubation, the cells were approximately 80% confluent. The complete growth medium was removed from all the wells and six replicates of appropriate concentrations of the test item extract (30, 40, 50, 60, 70, 80, 90 and 100%), neat extract of negative control (100%) and positive control (0.15 mg/mL) were added to their respective culture wells. The plate was then incubated at 37 °C with 5% CO_2 for 24 h. After 24 h of incubation, the cells were evaluated qualitatively (microscopic evaluation) to determine cell morphology and quantitatively (neutral red uptake method) to determine cell viability.

Qualitative evaluation

Under microscopic evaluation, the cultures treated with the negative control did not show any cytotoxic response (grade 0). Whereas the cells treated with positive control 0.15 mg/mL concentration showed severe cytotoxicity (grade 4). Therefore, the assay was considered valid.

The cultures treated with the test item extract at different concentrations appeared normal without any change in their morphology (grade 0) when compared with the negative control.

Quantitative evaluation

The assay was considered valid as the confluency of the cells before treatment was approximately 80%. The mean absorbance of cells in negative control was 0.376, the left and the right mean of the negative controls did not differ by more than 15% from the mean of all negative controls. The coefficient of variation (CV%) for the mean of replicate measurements were less than 15%. Positive control performed as expected (viability - 12.23%).

Cells treated with test item extract at different concentrations (30% to 100%) exhibited viability greater than 70% (ranging from 89.36% to 95.21%).

The percentage viability and cytotoxicity obtained for each concentration of the test item extract and the concurrent controls is given below.

Percentage viability and cytotoxicity											
	Negative Control	Test item extract concentrations (%)								Negative Control	Positive Control
		30	40	50	60	70	80	90	100		
Mean OD	0.378	0.358	0.354	0.349	0.350	0.343	0.344	0.338	0.336	0.374	0.046
SD (±)	0.006	0.005	0.007	0.007	0.009	0.007	0.008	0.007	0.007	0.007	0.007
CV (%)	1.6	1.4	2.0	2.0	2.6	2.0	2.3	2.1	2.1	1.9	15.2
Viability (%)	NA	95.21	94.15	92.82	93.09	91.22	91.49	89.89	89.36	NA	12.23
Cytotoxicity (%)	NA	4.79	5.85	7.18	6.91	8.78	8.51	10.11	10.64	NA	87.77

Based upon the results obtained in this study and in line with ISO 10993-5:2009(E), the given test item, Taglus Standard Thermoforming Foils supplied by Vedia Solutions, is non-cytotoxic to Balb/c 3T3 cells.

INTRODUCTION

Biocompatibility testing is a regulatory requirement for demonstrating the preclinical safety of medical devices. This is evaluated in line with the standard guideline, ISO 10993-1:2018(E), Biological Evaluation of Medical Devices - Part 1, Evaluation and Testing within a Risk Management Process. This standard describes the necessity to select a suitable test method for biocompatibility evaluation of medical devices.

Cytotoxicity assays are used to assess the effect of the device or its extract on cells grown *in vitro*. The elution method uses culture medium supplemented with serum as an extracting vehicle and are considered equivalent to the use of both polar and non-polar vehicles. The extracts are transferred onto a layer of cells and incubated for 24 hours. Following incubation, the cells are examined microscopically (qualitative) for their morphology, any malformation or degeneration, and cell lysis. In the quantitative assay, the neutral red (NR) uptake assay procedure is followed, which are based on the ability of viable cells to uptake neutral red dye. A reduction of >30% viability in the test item treated cultures compared to concurrent control culture indicates cytotoxicity.

OBJECTIVE

To evaluate the *in vitro* cytotoxic potential of the test item in Balb/c 3T3 cells using elution method.

Study Start Date	:	26 November 2021
Experiment Start Date (Cell line retrieval from liquid nitrogen)	:	30 November 2021
Experiment Completion Date	:	08 December 2021

STUDY DATES

The study completion date is the date the final report is signed by the Study Director.

TEST ITEM DETAILS

The test item, Taglus Standard Thermoforming Foils was received at GLR Laboratories Private Limited, on 23 October 2021 and stored at room temperature (20.1 to 24.1 °C) until used.

The following test item information provided by the sponsor, are considered an adequate description of the characterisation and stability of the test item.

Test Item	Taglus Standard Thermoforming Foils
Batch / Lot No.	12029092-1
Manufacture Date	29 September 2021
Expiry Date	20 September 2024
Appearance	Transparent disk
Ingredients	PETG (Polyethelene Tertamethylene Glycol)
Temperature Stability	37 °C
Sterility	Non-Sterile

The test item and control items were handled with all necessary protective clothing and all recommended safety and sterile measures were followed. Determinations of stability and characteristics of the test item were the responsibility of the Sponsor. No analysis was performed at GLR Laboratories Private Limited, to confirm it.

Description of the test item

The test item, Taglus Standard Thermoforming Foils is a surface device which comes in contact with mucosal membrane. The dimension of the test item are:- diameter: 12.5 cm and thickness: 0.8 mm. The duration of contact is less than 24 hours (limited).

DETAILS OF CONTROL ITEMS

Positive Control	Sodium Lauryl Sulphate (SLS); (Sigma - Aldrich, Batch no.: 0000009635; Expiry date: August 2022) in extraction medium (1x DMEM supplemented with 5% serum - DMEM 05, 4 mM L-glutamine and 1% Penicillin/ Streptomycin solution). This material has been routinely tested in GLR Laboratories Private Limited which consistently gives an excellent cytotoxic response with Balb/c 3T3 cells.
Negative Control	High-Density Polyethylene Film (RM-C) (Make: Hatano Research Institute, Food and Drug Safety Centre, Japan. Lot No.: C-211, Expiry Date: November 2027). HDPE was sterilized at 121 °C for 15 min before use.

TEST SYSTEM

Cell line	Balb/c 3T3, supplied by National Centre for Cell Science, India was cryopreserved in liquid nitrogen (-196 °C) until the commencement of the experiment. Vial no. P-7-1 was used for this experiment.
Growth conditions	Complete growth medium - DMEM 10 (Dulbecco's Modified Eagle Medium without L-Glutamine and with 25 mM HEPES (1x DMEM) (Lonza, Lot No. 0000966858; Expiry Date: January 2023) supplemented with 10% Heat Inactivated Newborn Calf Serum (Thermo Fisher Scientific, Lot No. 2212423; Expiry Date: April 2024), 4 mM L-glutamine (Thermo Fisher Scientific, Lot No. 2192423; Expiry Date: April 2022) and 1% Penicillin / Streptomycin solution (Lonza, Lot No. 20H055301; Expiry Date: August 2022). Antibiotics used does not adversely affect the assay. Extraction medium - DMEM 05 (Dulbecco's Modified Eagle Medium without L - Glutamine and with 25 mM HEPES (1x DMEM) supplemented with 5% Heat Inactivated Newborn Calf Serum, 4 mM L-glutamine and 1% Penicillin, Streptomycin solution).
Justification for use	Use of Balb/c 3T3 cells is recommended in ISO 10993, Part 5:2009(E) for assessing *in vitro* cytotoxicity.

TEST METHOD

Preparation of the test item extract

Rationale for selection of extraction ratio: Thickness of the test item is greater than 0.5 mm.

Extraction temperature and duration: 37 ± 1 °C for 24 h and 20 min.

The extraction details of the test and control item are given below:

Test/Control items Extracts	Extraction vehicle	Extraction ratio	Surface area/Weight (cm^2/g)	Volume of vehicle (mL)	Extraction start time	Extraction end time	Condition of extracts**
Test item	Extraction medium DMEM 05	3 cm^2/mL	245 cm^2	81.7	10:45 a.m. on 06 December 2021	11:05 a.m. on 07 December 2021	Pink clear solution without any particulates
Negative control		3 cm^2/mL	18 cm^2*	6.0			Pink clear solution without any particulates
Positive control	Extraction medium DMEM 05		0.15 mg/mL		Prepared before treating the cells on 07 December 2021		Pink clear solution without any particulates

* Both sides were involved in extraction.
**extraction vehicles did not undergo any colour changes during the extraction process.

No changes were observed in the retrieved test item. No additional processing such as filtration, centrifugation, pH adjustments or any other processing were made. The extract was used within 13 min of preparation and were considered stable during this time. This fulfils the requirement of ISO 10993-12:2012(E), ISO 10993-12:2021(E) and ISO 10993 5:2009(E). Eight different concentrations (30, 40, 50, 60, 70, 80, 90 and 100%) of the test item extract, neat extract of negative control (100%) and positive control (0.15 mg/mL) were prepared for the study.

Test procedure

Rationale for assay method

The NRU cytotoxicity assay procedure is a cell survival/viability chemosensitivity assay based on the ability of viable cells to incorporate and bind neutral red dye.

Specified in ISO 10993, Part-5:2009(E) standard as an appropriate test to evaluate *in vitro* cytotoxicity for assessing the biocompatibility of medical devices.

Exponentially growing Balb/c 3T3 cells were trypsinised using trypsin-EDTA (Make: Lonza, Lot No. 0000966852, Expiry date: January 2023) and counted in a hemocytometer using 0.4% Trypan blue (Lonza, Lot no. 0000847969, Expiry Date: March 2022). Exactly 1 x 10^5 cells per mL was prepared (0.839 mL of cell suspension [35.75 x 10^5 cells per mL] was added to 29.161 mL of culture medium to get 30 mL of cell suspension) and 100 µL was seeded in wells B2 to G12 of 96-well plate at a concentration

of 1 x 10^4 cells per well. The plates were then incubated with 5% CO_2 at 37 ± 1 °C for 24 h (06 December 2021, 10:04 a.m. to 07 December 2021, 10:04 a.m.).

The following day, confluency and morphology of the cells were observed and found to be approximately 80% confluent and normal. Then the complete growth medium was removed and six replicates of appropriate concentrations of the test item extract, positive control and negative controls were added to their respective culture wells as shown below:

96 - well plate template

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
B	Blank	Negative	Conc 1	Conc 2	Conc 3	Conc 4	Conc 5	Conc 6	Conc 7	Conc 8	Negative	Positive
C	Blank	Negative	Conc 1	Conc 2	Conc 3	Conc 4	Conc 5	Conc 6	Conc 7	Conc 8	Negative	Positive
D	Blank	Negative	Conc 1	Conc 2	Conc 3	Conc 4	Conc 5	Conc 6	Conc 7	Conc 8	Negative	Positive
E	Blank	Negative	Conc 1	Conc 2	Conc 3	Conc 4	Conc 5	Conc 6	Conc 7	Conc 8	Negative	Positive
F	Blank	Negative	Conc 1	Conc 2	Conc 3	Conc 4	Conc 5	Conc 6	Conc 7	Conc 8	Negative	Positive
G	Blank	Negative	Conc 1	Conc 2	Conc 3	Conc 4	Conc 5	Conc 6	Conc 7	Conc 8	Negative	Positive
H	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank

Blank: Medium Blank; Negative: Negative control, Positive: Positive control
Conc 1 to 8: Eight different concentrations of the test item extract - 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%, respectively
Alphabets A-H in the 96-well plate layout represents each row of the plate , Numbers 1-12 in the 96-well plate layout represents each column of the plate.

The plate was then incubated with 5% CO_2 at 37 ± 1°C for 24 h (07 December 2021, 11:18 a.m. to 08 December 2021, 11:18 a.m.). After 24 h of incubation, the cells were examined under inverted microscope for morphological evidence of cytotoxicity using a grading scheme according to ISO 10993-5:2009(E) (Table 1). Immediately following the visual assessment, wells were washed with 150 µL of phosphate buffered saline (PBS) (Lonza, Lot no. 0000955363, Expiry Date: December 2022) and 100 µL of neutral red medium was added. The plates were then incubated with 5% CO_2 at 37 ± 1°C for 3 h (08 December 2021, 11:40 a.m. to 02:40 p.m.).

Following incubation, the neutral red medium was removed and the cells were washed with 150 µL of PBS. Then 150 µL of neutral red desorb solution (ethanol: glacial acetic acid: distilled water, 10 mL:0.2 mL:9.8 mL) was added to the cells. Plates were shaken periodically until all neutral red was removed from the cells, forming a homogenous solution. The resulting coloured solution was analysed using a microplate reader (Multiskan sky) at a wavelength of 540 nm. Neutral Red absorbance was expressed in terms of absolute optical density (OD_{540}; which was OD_{540} of the culture minus the mean OD_{540} of medium blanks). Cell viability was calculated as the percentage of culture OD_{540} divided by negative control OD_{540}.

$$\text{Viability \%} = \frac{100 \times OD_{540e}}{OD_{540b}}$$

Where,
OD_{540e} is the mean value of the measured Optical Density of the test item;

OD_{540b} is the mean value of the measured Optical Density of the negative control.

The coefficient of variation (CV%) was calculated using the following formula:

$$CV\% = \frac{SD}{\text{Mean } OD_{540}} \times 100$$

ACCEPTANCE CRITERIA

The present assay is considered valid based on it meeting the following criteria:

1. Before treatment, cells had confluency of approximately 80%.
2. The left and the right mean of the negative controls did not differ more than 15% from the mean of all negative controls.
3. The mean absorbance value of negative control was ≥ 0.3.
4. The positive control showed a positive cytotoxic response of >30%.
5. The CV for replicate measurements was <15% except for positive control.

DATA EVALUATION

Qualitative evaluation: If the numerical grade obtained in qualitative evaluation is greater than 2 in the neat extract, then the test item is considered as cytotoxic.

Quantitative evaluation: If viability of the neat extract, as measured by neutral red uptake is less than 70%, compared to that of the negative control, then the test item is considered cytotoxic. Viability of greater than or equal to 70% indicates the test item is non-cytotoxic.

RESULTS

Before treatment, 80% cell confluency was observed in all wells.

Qualitative evaluation

Under microscopic evaluation, the cultures treated with the negative control did not show any cytotoxic response (grade 0). Whereas, the cells treated with positive control showed complete destruction of cell layers and was graded as severely cytotoxic (grade 4).

The cultures treated with the test item extract at different concentrations appeared normal without any change in their morphology (grade 0) when compared with the negative control.

Quantitative evaluation

The assay was considered valid as the confluency of the cells before treatment was approximately 80%. The mean absorbance of cells in negative control was 0.376 the left and the right mean of the negative controls did not differ by more than 15% from the mean of all negative controls. The coefficient of variation (CV%) for the mean of replicate measurements were less than 15%. Positive control performed as expected (viability - 12.23%).

Cells treated with test item extract at different concentrations (30% to 100%) exhibited viability greater than 70% (ranging from 89.36% to 95.21%).

CONCLUSION

Based upon the results obtained in this study and in line with ISO 10993-5:2009(E), the given test item, Taglus Standard Thermoforming Foils supplied by Vedia Solutions, is non-cytotoxic to Balb/c 3T3 cells.

REFERENCES

1. Biological Evaluation of Medical Devices - Part 1, Evaluation and Testing within a Risk Management Process, ISO 10993-1:2018(E).

2. Biological Evaluation of Medical Devices - Part 5, Tests for *in vitro* Cytotoxicity, ISO 10993-5:2009(E).

3. Biological Evaluation of Medical Devices - Part 12, Sample Preparation and Reference Materials, ISO 10993-12:2012(E).

4. Biological Evaluation of Medical Devices - Part 12, Sample Preparation and Reference Materials, ISO 10993-12:2021(E).

5. OECD Principles of Good Laboratory Practice. OECD Environmental Health and Safety Publications, Series on Principles of Good Laboratory Practice and Compliance Monitoring No. 1. ENV/MC/CHEM (98)17.

6. General Requirements for the Competence of Testing and Calibration Laboratories, ISO/IEC 17025:2017(E).

7. Use of International Standard ISO 10993-1, "Biological Evaluation of Medical Devices, ISO 10993 - Part 1. Evaluation and Testing Within a Risk Management Process. Guidance for Industry and Food and Drug Administration Staff. September 04, 2020.

Table 1: Qualitative Morphological Grading of Cytotoxicity of Extracts

Grade	Reactivity	Conditions of all cultures
0	None	Discrete intracytoplasmic granules, no cell lysis, no reduction of cell growth.
1	Slight	Not more than 20% of the cells are round, loosely attached and without intracytoplasmic granules, or show changes in morphology; occasional lysed cells are present; only slight growth inhibition observable.
2	Mild	Not more than 50% of the cells are round, devoid of intracytoplasmic granules, no extensive cell lysis; not more than 50% growth inhibition observable.
3	Moderate	Not more than 70% of the cell layers contain rounded cells or are lysed; cell layers not completely destroyed, but more than 50% growth inhibition observable.
4	Severe	Nearly complete or complete destruction of the cell layers.

Source: ISO 10993-5:2009(E)

Table 2: Qualitative scoring for cytotoxicity

	Blank	Neg	30%	40%	50%	60%	70%	80%	90%	100%	Neg	Pos
	1	2	3	4	5	6	7	8	9	10	11	12
A	No cells	No cells	No cells	No cells	No cells	No cells	No cells	No cells	No cells	No cells	No cells	No cells
B	No cells	0	0	0	0	0	0	0	0	0	0	4
C	No cells	0	0	0	0	0	0	0	0	0	0	4
D	No cells	0	0	0	0	0	0	0	0	0	0	4
E	No cells	0	0	0	0	0	0	0	0	0	0	4
F	No cells	0	0	0	0	0	0	0	0	0	0	4
G	No cells	0	0	0	0	0	0	0	0	0	0	4
H	No cells	No cells	No cells	No cells	No cells	No cells	No cells	No cells	No cells	No cells	No cells	No cells

Blank: Medium; Neg: Negative control; Pos: Positive control. 0, None; 1, Slight; 2, Mild; 3, Moderate; and 4, Severe cytotoxicity

Table 3: Optical density readings at 540 nm

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.080	0.089	0.078	0.090	0.071	0.080	0.076	0.082	0.085	0.079	0.088	0.081
B	0.072	0.466	0.444	0.436	0.430	0.445	0.429	0.425	0.431	0.407	0.456	0.137
C	0.074	0.463	0.432	0.445	0.439	0.430	0.426	0.433	0.414	0.414	0.460	0.132
D	0.084	0.457	0.441	0.437	0.425	0.421	0.417	0.428	0.415	0.422	0.463	0.123
E	0.075	0.450	0.434	0.432	0.433	0.428	0.431	0.422	0.411	0.419	0.450	0.117
F	0.073	0.461	0.442	0.425	0.427	0.422	0.419	0.427	0.416	0.411	0.451	0.121
G	0.079	0.452	0.437	0.430	0.418	0.431	0.414	0.410	0.420	0.424	0.445	0.126
H	0.067	0.086	0.082	0.091	0.083	0.070	0.083	0.076	0.087	0.084	0.069	0.078

Mean of media blanks: 0.080

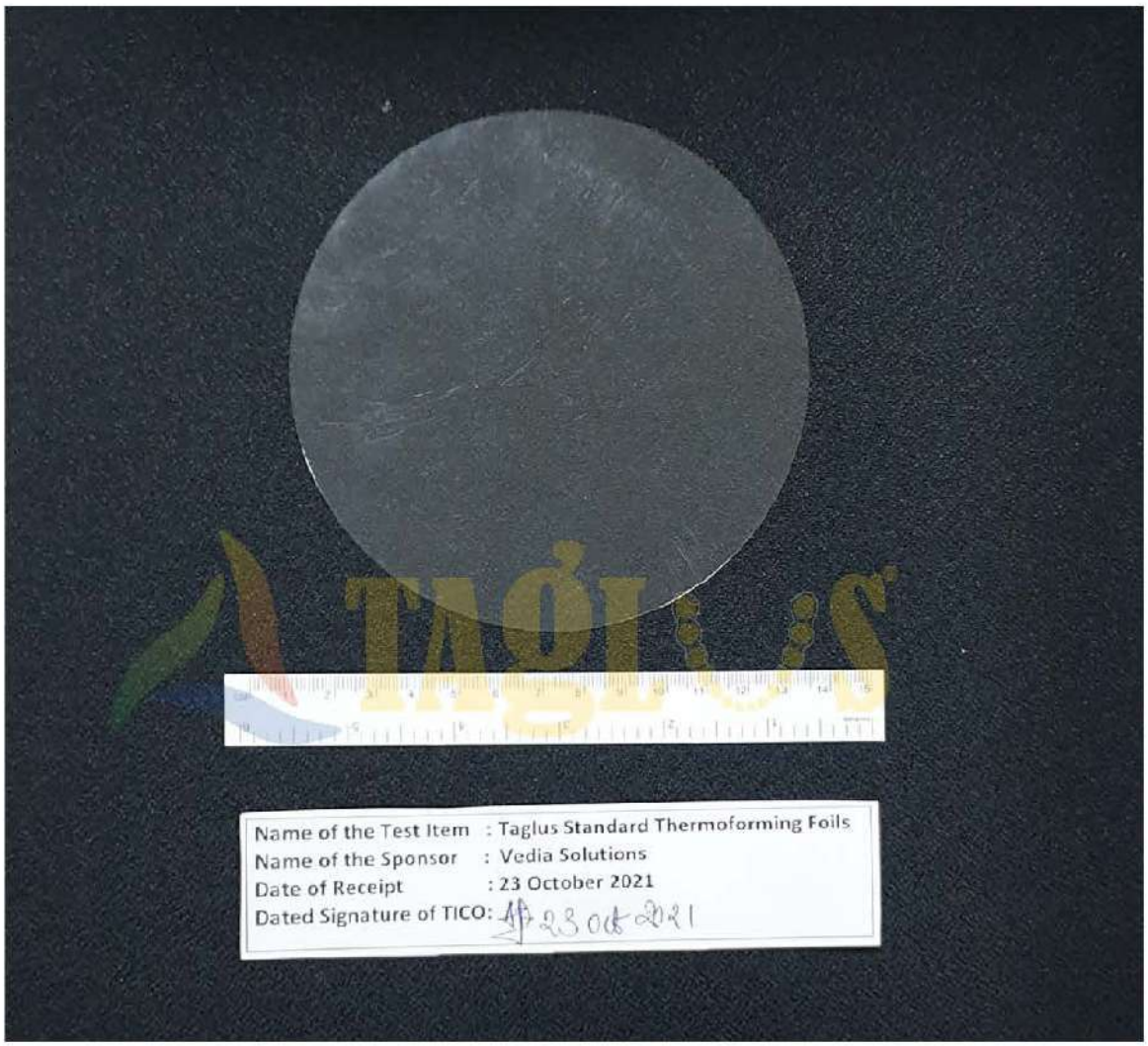
Table 4: ODs adjusted with media blank

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0	0	0	0	0	0	0	0	0	0	0
B	0	0.386	0.364	0.356	0.350	0.365	0.349	0.345	0.351	0.327	0.376	0.057
C	0	0.383	0.352	0.365	0.359	0.350	0.346	0.353	0.334	0.334	0.380	0.052
D	0	0.377	0.361	0.357	0.345	0.341	0.337	0.348	0.335	0.342	0.383	0.043
E	0	0.370	0.354	0.352	0.353	0.348	0.351	0.342	0.331	0.339	0.370	0.037
F	0	0.381	0.362	0.345	0.347	0.342	0.339	0.347	0.336	0.331	0.371	0.041
G	0	0.372	0.357	0.350	0.338	0.351	0.334	0.330	0.340	0.344	0.365	0.046
H	0	0	0	0	0	0	0	0	0	0	0	0

Table 5: Viability and cytotoxicity percentage

Percentage viability and cytotoxicity											
	Negative Control	Test item extract concentrations (%)								Negative Control	Positive Control
		30	40	50	60	70	80	90	100		
Mean OD	0.378	0.358	0.354	0.349	0.350	0.343	0.344	0.338	0.336	0.374	0.046
SD (±)	0.006	0.005	0.007	0.007	0.009	0.007	0.008	0.007	0.007	0.007	0.007
CV (%)	1.6	1.4	2.0	2.0	2.6	2.0	2.3	2.1	2.1	1.9	15.2
Viability (%)	NA	95.21	94.15	92.82	93.09	91.22	91.49	89.89	89.36	NA	12.23
Cytotoxicity (%)	NA	4.79	5.85	7.18	6.91	8.78	8.51	10.11	10.64	NA	87.77

PHOTOGRAPH OF THE TEST ITEM



RESPONSIBLE PERSONNEL

Mr. V. Rajasekar, MTech (Biotech)	Study Director
Ms. P. Pradeepa, MPhil, ERT	Study Scientist
Mr. R. Arun, MSc	Study Scientist
Ms. S. Koezhily, MSc	Study Scientist

STATEMENT OF STUDY COMPLIANCE

The study was performed in compliance with:

- OECD Principles of Good Laboratory Practice (revised 1997, issued January 1998) ENV/MC/CHEM (98) 17.
- US Food and Drug Administration's GLP regulations, 21 CFR Part 58 (subparts B to G and J).
- ISO/IEC 17025:2017(E) (general requirements for the competence of testing and calibration laboratories).

All procedures were performed in accordance with GLR Laboratories Private Limited standard operating procedures (SOPs). The study was subjected to Quality Assurance evaluation by the GLR Laboratories Private Limited Quality Assurance Unit (QAU) in accordance with SOPs.

STUDY PLAN AMENDMENT

No study plan amendment was made during the conduct of the study.

STUDY PLAN DEVIATION

No study plan deviation occurred during the conduct of the study.

ARCHIVE STATEMENT

All primary data, or authenticated copies thereof, a sample test item, study plan and the final report will be retained for a period of 9 years in the GLR Laboratories Private Limited archives, after issue of the final report. At the end of the specified archive period the Sponsor will be contacted to determine whether the data should be returned, retained or destroyed on their behalf. Sponsors will be notified of the financial implications of each of these options at that time.

DISTRIBUTION OF REPORTS

Two originals of the study report are prepared and distributed as mentioned below:

1. Sponsor.
2. Archive (GLR Laboratories Private Limited).

ANNEXURE 1

सत्यमेव जयते

GOVERNMENT OF INDIA
Department of Science and Technology
National Good Laboratory Practice (GLP) Compliance Monitoring Authority (NGCMA)

Certificate of GLP Compliance

This is to certify that

GLR Laboratories Private Limited
444, Gokulam Street, Mathur
Madhavaram, Chennai-600068 (Tamil Nadu), India

is a GLP certified test facility in compliance with the NGCMA's Document No. GLP-101 "Terms & Conditions of NGCMA for obtaining and maintaining GLP certification by a test facility" and OECD Principles of GLP.

The test facility conducts the below-mentioned tests/ studies:

- **Toxicity Studies**
- **Mutagenicity Studies**

The specific areas of expertise, test items and test systems are listed in the annexure overleaf.

Validity: March 13, 2020 – April 3, 2022

Certificate No. : GLP/C-132A/2020
Issue Date : 13-03-2020

(Dr. Neeraj Sharma)
Head, NGCMA