Consultancy and Laboratory Services for Biomedical Quality

CYTOTOXICITY TEST

REPORT N. 00523-21 Rev. 00

Customer: STUDIO DI MICROBIOLOGIA ED ECOLOGIA SRL
Via delle Anime, 87 - 73100 Lecce

TEST METHOD ISO 10993-12:2012 + ISO 10993-5:2009 Annex A

TIME SCHEDULE

Acceptance N.:	21-00079
Reception date:	07/01/2021
Start test date:	20/01/2021
End test date:	22/01/2021
Operator:	Dr. E. Fabbri

TEST SAMPLE IDENTIFICATION

Name:	Mascherina facciale
Sample Typology:	Mascherina chirurgica
Composition:	not provided
Device Category:	NOT PROVIDED
Quantity tested:	1
Code (REF):	9.358_20
LOT:	not provided
Manufacturing date:	not provided
Expiry date:	not provided
Sterilization Method:	Not sterile
Sterilization lot:	n/a
Sterilization Date:	n/a
Sterilization Unit:	n/a

The information concerning the test sample were provided by the Customer. All data related to the test sample are under the responsibility of the Customer and have not been verified by the test laboratory.

Issue Date	Rev.	Change Description	Prepared by: Dr. E. Fabbri (Laboratory Technician)	Verified and Approved by: Dr. Renzo Giovanni Coronati (Managing Director Laboratory)
27/01/2021	00	First Issue	Elisa Fabbri	R Coronati

This test report is digitally signed by Dr. Renzo Giovanni Coronati.
The digital signature has legal value according to Italian D. Lgs. 82/2005 and subsequent amendments.

The sampling is performed by the Customer. The test results relate only to the test sample as received.
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LAB N° 1496 L

PROCEDURES
All procedures used during this study are recorded in the Laboratory Coronati Consulting s.r.l.

OPERATING METHOD – (test on extracts)
Test Sample preparation
Under laminar flow hood in aseptic conditions, the device (without elastic bands) was put in contact with extracting solution (DMEM +NBCS). The quantity of extracting solution was selected according to the ratio of 0.1 g/ml (sample/extracting solution) required by the standard UNI EN ISO 10993-12. The sample has been maintained in contact with the extract solution at 37 ± 1°C for 24 hours and continuously stirred required by the standard UNI EN ISO 10993-12. Before the contact with the cells, the extract was submitted to filtration.

Negative control preparation
The extract of the negative control was prepared by immersing HDPE in culture medium, in order to reach a surface/volume ratio of 3 cm^2/ml. Then the test sample was incubated for 24 hours at temperature of 37 ± 1°C
Positive control preparation
The extract of the positive control was prepared by immersing latex in culture medium, in order to reach a surface/volume ratio of 6 cm^2/ml. Then the test sample was incubated for 24 hours at temperature of 37 ± 1°C.
Treatment
The test has been carried out using the cellular line BALB 3T3 clone A31 (fibroblasts from mouse embryo) that has a high capacity to proliferate (as recommended in UNI EN ISO 10993-5). Cell cultures were grown until a confluent monolayer in multiwell dishes. Sample extract in 6 replicates has replaced the medium in dishes containing cell cultures and it was incubated at 37 ± 1°C for 24 hours in at 10% CO_2 atmosphere. Negative control and positive control were prepared at the same time and submitted to the same process of the sample. At the end of incubation the cells were treated for 3 hours with a solution containing Neutral Red and by photometer the colouring intensity (proportional to cellular vitality) was measured.

REFERENCE CONTROLS
- HDPE – High Density PolyEthylene (Negative Control) (USP)
- Latex from glove (Positive Control) (VWR International S.r.l)

INSTRUMENTS AND EQUIPMENT
- Laminar flow hood (ATR 034)
- Stirrer with thermostatic cupola (ATR 066)
- CO_2 Incubator for cultures cell (ATR 156)
- Inverted Microscope (ATR 032)
- Photometer Microplate Reader (ATR 193)

ACCEPTANCE CRITERIA
- Mean of OD_{540} of negative control replicates must be ≥ 0,3 **(1)**.
- Vitality of positive control must be ≤ 50% **(1)**.
- C.V.% between replicates must be ≤ 20%.

(1) according to UNI EN ISO 10993-5:2009

INTERPRETATION OF RESULTS
- Sample is considered non cytotoxic if cellular vitality is ≥ 70%.
- Sample is considered cytotoxic if the cellular vitality is < 70%.

RESULTS

Mean of OD_{540} of Negative Control replicates		≥ 0,3	
Id.	Cellular Vitality %	Uncertainty estimated with factor K=2 (95%)	C.V.%
Sample Extract	**92,1**	**± 4,5**	5,5
Negative Control	100,0	**± 4,8**	6,8
Positive Control	19,2	**± 1,5**	4,3

CONFORMITY EVALUATION
Under the assay conditions the extract of the sample is considered NON-CYTOTOXIC, take into account the uncertainty associated with the percentage of cellular vitality.

----------*End of Report*----------

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