Consultancy and Laboratory Services for Biomedical Quality

CYTOTOXICITY TEST

REPORT N. 8419-20 Rev. 00

Customer: IDEANTIS SRL
VIALE MONTE NERO 80 - 20135 - MILANO

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

TIME SCHEDULE

Acceptance N.:	20-7996
Reception date:	26/11/2020
Start test date:	30/11/2020
End test date:	02/12/2020
Operator:	Dr. E. Fabbri

TEST SAMPLE IDENTIFICATION

Name:	Maskèdra
Sample Typology:	Mascherina chirurgica
Composition:	poliuretano+poliammide12+Spunbond+Melt blown+natural rubber+poliestere (For additional information see Annex 01)
Quantity tested:	1
Code (REF):	N/A
LOT:	001
Manufacturing date:	ottobre 2020
Expiry date:	Non applicabile
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)
11/12/2020	00	First Issue	Elisa Fabbri	

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The digital signature has legal value according to Italian D. Lgs. 82/2005 and subsequent amendments.

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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 mask (without elastic bands) and the filter were put in contact with extracting solution (DMEM +NBCS). The quantity of extracting solution for the filter was selected according to the ratio of 0.1 g/ml (sample/extracting solution), instead, for the mask according to the ratio of 0.2 g/ml (sample/extracting solution) required by the standard UNI EN ISO 10993-12. The mask and the filter have been maintained in contact with the extract solution at 37 ± 1°C for 24 hours and continuously stirred. Before the contact with the cells, the extracts were submitted to the sterilizing 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. The two extracts from the filter and from the mask, formed a single device, were mixed. The mixture obtained, 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	**100,0**	**± 4,8**	8,0
Negative Control	100,0	**± 4,8**	2,5
Positive Control	10,3	**± 1,2**	3,4

CONFORMITY EVALUATION

Under the assay conditions the extract of the sample is considered NON-CYTOTOXIC.

DEVIATION

No deviation has been remarked during the study.

ANNEXES

Annex 01: Sample Composition and Drawing Sample

End of Report

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