

CYTOTOXICITY TEST

REPORT N. 06530-21 Rev. 00

Customer: **SC EURO IMPACT Srl**
Strada Principale, Ruscova, NO. 698 - Judetul Maramures

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

TIME SCHEDULE

Acceptance N.:	21-05557
Reception date:	09/09/2021
Start test date:	22/09/2021
End test date:	24/09/2021
Operator:	Dr. E. Fabbri

TEST SAMPLE IDENTIFICATION

Name:	DISPOSABLE MEDICAL MASK ELIMEDICAL
Sample Typology:	MEDICAL MASK
Composition:	Polypropylene nonwoven , Non-woven filter fabric Meltblown, 25g m ² , elastic 3mm, nose wire 3mm
Device Category:	< 24 hours
Quantity tested:	1
Code (REF):	not provided
LOT:	A1-98-01.09.2021
Manufacturing date:	01.09.2021
Expiry date:	36 MONTHS FROM THE DATE OF PRODUCTION
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)
30/09/2021	00	First Issue		

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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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 immersed in extracting solution (DMEM+5% NBCS+Antibiotics). The quantity of extracting solution was selected according to the ratio of 0.1 g/ml (sample/extracting solution) required by the standard ISO 10993-12. The sample has been maintained in contact with the with extracting solution at $(37 \pm 1)^{\circ}\text{C}$ for (24 ± 2) h and continuously stirred required by the standard ISO 10993-12.

Nothing abnormal was detected as regards the extract appearance: no signs of particle, clouding, discoloring or chemical precipitation were recorded; the extract was not diluted and centrifuged before use. No pH adjustment was made in the extract before use. Before the contact with the cells, the extract was submitted to filtration.

Extract was used immediately after preparation.

0,1 ml of the extract was pipetted in each well with cells (6 replicates).

Negative control preparation

The extract of the negative control was prepared by immersing HDPE in extracting solution (DMEM+5% NBCS+Antibiotics), in order to reach a surface/volume ratio of $3 \text{ cm}^2/\text{ml}$. Then the test sample was incubated at $(37 \pm 1)^{\circ}\text{C}$ for (24 ± 2) h.

0,1 ml of the extract was pipetted in each well with cells (6 replicates).

Positive control preparation

The extract of the positive control was prepared by immersing latex in extracting solution (DMEM+5% NBCS+Antibiotics), in order to reach a surface/volume ratio of $6 \text{ cm}^2/\text{ml}$. Then the test sample was incubated at $(37 \pm 1)^{\circ}\text{C}$ for (24 ± 2) h.

0,1 ml of the extract was pipetted in each well with cells (6 replicates).

Vehicle Control

Extracting solution (DMEM + 5% NBCS+Antibiotics) not containing the test sample was incubated at $(37 \pm 1)^{\circ}\text{C}$ for (24 ± 2) h. 0,1 ml of the extract was pipetted in each well with cells (6 replicates in the left column and 6 replicates in the right column).

Blank

Extracting solution (DMEM + 5% NBCS+Antibiotics) not containing the test sample was incubated at $(37 \pm 1)^{\circ}\text{C}$ for (24 ± 2) h. 0,1 ml of the extract was pipetted along the entire perimeter of the well without cells.

Treatment

The test was carried out using the cellular line BALB 3T3 clone A31 (fibroblasts from mouse embryo) that has a high capacity to proliferate (as recommended in ISO 10993-5:2009 Annex A). Cell cultures were grown until a confluent monolayer in multi-well dishes. Sample extract in 6 replicates has replaced the medium in dishes containing cell cultures and it was incubated at $37 \pm 1^{\circ}\text{C}$ for 24 hours in at 10% CO_2 atmosphere. Negative control, Positive control and Vehicle 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 OF THE METHOD

- Mean of OD_{540} of Vehicle Control replicates must be $\geq 0,3$ ⁽¹⁾.
- Vitality of Positive Control must be $\leq 50\%$ ⁽¹⁾.
- Vitality of Negative Control must be $\geq 70\%$ ⁽¹⁾.
- Coefficient of Variation % (C.V.%) between replicates must be $\leq 20\%$.

⁽¹⁾ according to ISO 10993-5:2009

INTERPRETATION OF RESULTS

- Sample is considered non cytotoxic if cellular vitality is $\geq 70\%$.
- Sample is considered cytotoxic if the cellular vitality is $< 70\%$.

RESULTS

All acceptance criteria of the method are valid.

Id.	Cellular Vitality %	Uncertainty estimated with factor K=2 (95%)	C.V.%
Sample Extract	85,1	$\pm 4,3$	4,6
Negative Control	90,7	$\pm 4,5$	5,5
Positive Control	11,5	$\pm 1,3$	3,2
Vehicle Control	100,0	$\pm 4,9$	6,0

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-----