Analytical Report: AAL44185
Eurofins Sample Number: LV20AB9066-1
Version: 1

LAB N° 0032 L

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Client Account Number: A00895229RF5
Eurofins Quote Number: PO9YPH20081401

Eurofins Sample Number LV20AB9066-1	
Original Received Date:	24-Nov-2020
Description:	§ 3ply non woven disposable surgical masks
Lot Number:	§ L202891

Analysis	Result	Unit
# In vitro cytotoxicity		
RESULT:	Not cytotoxic	----
Test item:	30mm²	----
Vehicle:	Routine medium	----
Positive control:	30mm² of Latex	----
Negative control:	30mm² of HDPE	----
Notes:	N/A	----

Addendum #1: Qualitative and quantitative evaluation

Method: ISO 10993-5:2009
Analysis Date: 02-Dec-2020 to 04-Dec-2020

Supplemental Information

For LV20AB9066-1:

Sample description: green surgical mask

Storage: room temperature

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LAB N° 0032 L

Contracted Company: Eurofins Biolab Srl (Vimodrone)

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Reviewed and electronically signed for Technical Supervisor Approval by
Elena Bizzotto, Employee
for Eurofins Biolab Srl, on 09-Dec-2020 16:21:45 UTC+01:00

eurofins	In vitro cytotoxicity ISO10993-5:2009	1-P-QM-TEM-9070229
	Test by Direct Contact - NRU	Addendum N. 1

Study ID: //

Sample ID: LV20AB9066-1

Test start: 02/12/2020

Test end: 04/12/2020

Cell line	Manufacturer
Mammal fibroblasts BALB/3T3 clone A31 (ATCC® CCL163™)	American Type Culture Collection (ATCC)

Reagent	Manufacturer	Batch
Dulbecco's Modified Eagle Medium (DMEM)	Sigma-Aldrich	RNBJ2369
Fetal Bovine Serum (FBS)	Sigma-Aldrich	17H114
Penicillin/Streptomycin solution	Sigma-Aldrich	0000088375

QUALITATIVE EVALUATION

	Contact time: 24 h			
	PLATE 1			
	Blank	Vehicle control	Negative control	Test Sample
Replicate 1		0	0	0
Replicate 2		0	0	0
Replicate 3		0	0	0

	Contact time: 24 h			
	PLATE 2			
	Blank	Vehicle control	Positive control	Test Sample
Replicate 1		0	4	0
Replicate 2		0	4	0
Replicate 3		0	4	0

INTERPRETATION OF RESULT

Grade	Reactivity	Conditions of all Cultures
0	None	No detectable zone around or under specimen
1	Slight	Some malformed or degenerated cells under specimen
2	Mild	Zone limited to area under specimen
3	Moderate	Zone extending specimen size up to 1.0 cm
4	Severe	Zone extending farther than 1.0 cm beyond specimen

ACCEPTANCE CRITERIA

Negative control	
Grade ≤1	VALID

Positive control	
Grade ≥3	VALID

RESULTS

	Reactivity grade
Test Sample	0

eurofins	In vitro cytotoxicity ISO10993-5:2009	1-P-QM-TEM-9070229
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Study ID: // Sample ID: LV20AB9066-1

Test start: 02/12/2020 Test end: 04/12/2020

QUANTITATIVE EVALUATION - 540 nm

	Contact time: 24 h			
	PLATE 1			
	Blank	Vehicle control	Negative control	Test Sample
Replicate 1	0,048	1,496	1,463	1,486
Replicate 2	0,051	1,442	1,217	1,454
Replicate 3	0,046	1,501	1,491	1,479

	Contact time: 24 h			
	PLATE 2			
	Blank	Vehicle control	Positive control	Test Sample
Replicate 1	0,047	1,464	0,046	1,424
Replicate 2	0,048	1,434	0,040	1,442
Replicate 3	0,045	1,458	0,042	1,433

MEAN, STANDARD DEVIATION, CV% AND VIABILITY

	Mean OD	Standard Deviation	CV %	Mean OD - Mean OD_{Blanks}	Viability %
Blanks	0,048				
Vehicle control	1,466	0,028	1,879	1,418	100
Negative control	1,390	0,151	10,844	1,343	95
Positive control	0,043	0,003	7,160	-0,005	0
Test Sample	1,453	0,025	1,721	1,406	99

ACCEPTANCE CRITERIA

	Vehicle ≥ 0,3
Mean OD	VALID

	Negative control ≥ 70%	Positive Control < 70%
Quantitative Evaluation	VALID	VALID

	Vehicle control	Negative control	Positive control	Test Sample
CV between replicates ≤ 15%	VALID	VALID	VALID	VALID

$$\%\,viability = \frac{OD_{\underset{sample}{mean}} - OD_{\underset{blank}{mean}}}{OD_{\underset{vehicle}{mean}} - OD_{\underset{blank}{mean}}} \times 100$$

INTERPRETATION OF RESULTS

Reduction of Viability	Result
≤ 30%	Not Cytotoxic
> 30%	Cytotoxic

RESULTS

	Reduction Viability %	
Test Sample	1	NOT CYTOTOXIC

*0 (% viability test item ≥ vehicle); 100 (% viability test item ≤ Blank)

Finished on: 04/12/20 Technician signature: [signature]

Date: 09/12/20 Approved by: [signature]

Documento completo appovato in ETQ

eurofins | Medical Device Testing	Test Facility Eurofins Biolab S.r.l.	Sample ID: Page: 1 of 1

ADDENDUM N.2: EXPERIMENTAL REPORT

REFERENCE/GUIDELINE:	- ISO 10993-5:2009 - In vitro cytotoxicity	
CELL LINE	Mammal fibroblasts BALB/3T3 clone A31 (ATCC®; CCL163™) Source: ATCC.	
MATERIALS	Foetal Bovine Serum (FBS), Trypan Blue, Neutral Red dye, SDS, Penicillin/Streptomycin solution, Dulbecco's Modification of Eagle's Medium (DMEM), Dulbecco's Phosphate buffer solution (DPBS)	Sigma-Aldrich
	Trypsin-EDTA	Lonza
	Acetic Acid, Ethanol	VWR
	Water for Injection	Eurospital
	High density polyethylene (HDPE, USP Reference Standard negative control)	Nova Chimica (KOM357)
	Latex from laboratory gloves	Artsana (1811191C170)
EQUIPMENT	Laminar flow hood, CO_2 incubator, Microplate reader Mod EL800, Chronometer, Common laboratory equipment, Water, Inverted Microscope Diavert,Orbital shaker, Refrigerator	

EXPERIMENTAL DESIGN

The experimental design included two 12-well plate containing a subconfluent cell monolayer subdivided in the following groups:

GROUPS	REPLICATES PLATE N.1			REPLICATES PLATE N.2		
	Blank	Blank	Blank	Blank	Blank	Blank
	Vehicle	Vehicle	Vehicle	Vehicle	Vehicle	Vehicle
	Negative control	Negative control	Negative control	Positive control	Positive control	Positive control
	Test sample	Test sample	Test sample	Test sample	Test sample	Test sample

BLANK	Supplemented culture medium alone (without cells).
VEHICLE	Supplemented culture medium (without test sample).
TEST SAMPLE	**30 mm²** of the test sample were placed in the middle of each well.
NEGATIVE CONTROL	The negative control was represented by **30 mm²** of HDPE placed in the middle of each well.
POSITIVE CONTROL	The positive control was represented **30 mm²** of latex placed in the middle of each well.

TREATMENT: Verified that a subconfluent monolayer was present, supplemented culture medium was replaced and the test sample was added. The plates were incubated in a thermostat at 37°C ±1°C in a 5% CO_2 atmosphere for 24 hours.
This procedure was repeated for positive and negative controls.

QUALITATIVE EVALUATION (GRADE OF CYTOTOXICITY): After 24 hours the plates were observed under an inverted microscope and biological reactions were evaluated following a 0 to 4 scale according to ISO10993-5:2009.

QUANTITATIVE EVALUATION (OPTICAL DENSITY): After microscopic observation, cells were treated with Neutral Red Medium for 3 hours at 37°C ±1°C in 5% CO_2 atmosphere. Subsequently, the Neutral Red medium was removed and each well was rinsed with DPBS. The plates were totally made dry reversing the plates, then Desorb Solution was added and the plates were incubated for 10 minutes at room temperature with gentle agitation to form a homogeneous solution. Optical density was measured at 540nm by Gen5 software (Biotek) using microtiter plate reader.

$$\%\ \text{of cell viability} = \frac{\text{OD test sample} - \text{OD blank}}{\text{OD vehicle} - \text{ODblank}} \cdot 100$$

ACCEPTABILITY CRITERIA	QUALITATIVE EVALUATION	Negative control ≤ 1; Positive control ≥ 3
	QUANTITATIVE EVALUATION	The OD mean of the vehicle must be ≥ 0,3. The positive control % cellular viability must be < 70%. The negative control % cellular viability must be ≥ 70%. Coefficient of variation of each group must be ≤15%.
INTERPRETATION OF RESULTS	The achievement of a numerical grade greater than 2 is considered a cytotoxic effect. A cellular viability reduction more than 30% is considered a cytotoxic effect.	
QUALITY CRITERIA	Satisfied	
QUANTITATIVE EVALUATION	Cells treated with test sample have shown a cell viability reduction of **1±0,76%.**	

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