Ontex bv
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Your notice of	**Your reference**	**Date**
31-08-2020		05-10-2020

Analysis Report 20.05211.02

Required tests :

EN 14683 (2019) + AC (2019)	**EN 14683 - §5.2.5 (2019) AC (2019)**	**Microbial cleanliness on masks**
	ISO 10993-5 (2009)	**Cytotoxicity**

Sample id	Information given by the client	Date of receipt
T2018458	SMtIIR-010	31-08-2020

Sylvie Niessen
Order responsible

The results of the analysis cover the received samples. Centexbel is not responsible for the representativeness of the samples.
In assessing compliance with the specifications, we did not take into account the uncertainty on the test results.

Reference: **T2018458 - SMtIIR-010**

Microbial cleanliness on masks

Date of ending the test	02-10-2020
Standard used	EN 14683 - §5.2.5 (2019) AC (2019)
Product standard	EN 14683 (2019) + AC (2019)
Number of tested masks	5
Extraction liquid	Peptone 1g/l, NaCl 5g/l & Tween 20 2g/l
Extraction volume	300 ml
Extraction time	5 min.
Counting technique	Membrane filtration
Filtration volume	100 ml
Culture media	TSA (Tryptic Soy Agar) SDA (Sabouraud Dextrose Agar with chloramphenicol)
Incubation conditions	3 days at 30°C (TSA) 7 days at 20-25°C (SDA)

Results

# Mask	Mask weight (g)	CFU*/mask		Microbial cleanliness	
		Aerobic microbial count (bacteria)	*Fungi count (SDA)*	*Σ CFU/mask*	*Σ CFU/g*
1	3.81	15	< 3	< 18	< 5
2	3.78	12	6	18	5
3	3.78	33	3	36	10
4	3.75	9	< 3	< 12	< 4
5	3.77	9	3	12	4

Note :

The performance requirements for medical face masks according to EN 14683 (2019) + AC (2019) is :

Test	Type I	Type II	Type IIR
Microbial cleanliness (cfu/g)	≤ 30	≤ 30	≤ 30

Performed under accreditation in the microbiological lab under the responsibility of Yvette Rogister

Reference: **T2018458 - SMtIIR-010**

Cytotoxicity

Date of ending the test	30-09-2020
Standard used	ISO 10993-5 (2009)

1. Method

ISO 10993-5: 2009 – Biological evaluation of medical devices – Part 5: Tests for *in vitro* cytotoxicity

Test method used : Test on extracts and Measurement of cell viability by XTT Assay

Principle :

Monolayers of *in vitro* cultured cells are incubated for 24 hours at 37°C and 5 % CO_2 in the presence of the device or material extract. After incubation , the cytotoxic effect of the device or material is determined by assessing the cell viability using the XTT assay.
Next to the device or material under investigation, negative and positive controls are simultaneously checked.
If the sample extract shows a mean cell viability ≥70 %, the sample is considered as non-cytotoxic.

2. Results

The test has been performed by GD from 28.09.2020 to 30.09.2020

General information

Cells :	Mouse fibroblasts
Strain :	*L929 (ATCC CCL-1, NCTC Clone 929)*
Passage :	*585*
Sample description :	White non-woven mask
Sterilisation of the sample :	UV (10 min. each side)
Extraction medium :	Complete Dulbeccos' Modified Eagle's Medium (Dulbeccos' Modified Eagle's Medium (Lonza, lot 718751) supplemented with 10%FBS (VWR, Lot S18092S181H) and 1% Penicillin/ Streptomycin /Amphotericin B (Lonza, lot 19B135304)

Extraction ratio *(according to ISO 10993-12)*	0.1 g/ ml (ratio recommended for textiles)
Extraction conditions :	24 hours under agitation at 37°C
Test procedure :	Incubation of the cells in the presence of the extract(s) and controls for 24h at 37°C
Reagent control :	Extraction medium (without test material) that has been subjected to the same extraction conditions as for the sample.
Positive control	Solution of Triton X100 (in complete DMEM)
Negative control	HDPE film

Cytotoxicity assessment:

After the 24 h incubation of the cells with the extract (or control), the XTT (2,3-Bis-(2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxyanilide salt) solution is added. After incubation for 2 hours at 37°C (5% CO_2), the resulting solution is measured at the spectrophotometer (OD_{450nm}).
XTT is a salt that is cleaved to formazan by the succinate dehydrogenase system of the mitochondrial respiratory chain of the cells.
An increase or decrease in cells number results in a concomitant change in the amount of formazan formed, indicating the degree of cytotoxicity caused by the test material. The cell viability is expressed as a percentage of the reagent control.

samples	cell viavibility % (± Std Dev)
Reagent control (100% viability)	100.00 ± 8.55
T2018458 (100 %)	**88.61 ± 11.04**
T2018458 - 50 % (= diluted 2 times)	86.01 ± 11.29
T2018458 - 25 % (= diluted 4 times)	87.43 ± 11.80
T2018458 - 13 % (= diluted 8 times)	94.07 ± 5.20
T2018458 - 6 % (= diluted 16 times)	90.79 ± 3.16
T2018458 - 3 % (= diluted 32 times)	95.13 ± 9.52

The reagent control, positive control and negative control performed as anticipated.

3. Conclusions

According to ISO 10993-5, a cell viability equal or higher than 70% is considered as non-cytotoxic.
Under the conditions of the assay, the tested sample **T2018458** has therefore to be considered as **non-cytotoxic**.