TESTS FOR IN VITRO CYTOTOXICITY

Test Substance

01 CSAFE01 XX COVERSAFE™

Test Report N°20-0754-01-I

Test performed for

GERGONNE INDUSTRIE
ZI Nord - Rue de Tamas - CS 70204
01117 OYONNAX CEDEX - FRANCE

by
BIOCHEM S.r.l.
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QUALITY ASSURANCE

Quality Assurance Manager: Alessandra Marchesi, PhD

TEST DIRECTOR

Giovanni Bassini, Ch.Eng.

TIME SCHEDULE OF TEST

The test was started on 16/06/2020 and was completed on 18/06/2020.

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Ref. Your Order PO193228 of 11/06/2020

Sample description

Denomination: 01 CSAFE01 XX COVERSAFE™
Code: /
Lot: /
Sterilization: No
Receipt number: 16556
Receipt date: 15/06/2020
Sampling carried out by: GERGONNE INDUSTRIE

Part of the sample to be tested: The whole sample
Pretreatment: /

Test Method

ISO 10993-5: 2009
ISO 10993-12: 2012

Other references

Cytotoxicity Test Protocol - /

Summary of practice

Cell cultures are grown to a near-confluent monolayer in cultures dishes. Three dishes for each sample are prepared. Moreover, three dishes are prepared for the Negative control, three for the Positive control and three for the Cell Culture Medium (MEM control). A portion/aliquot of the test sample, of the negative control and of the positive control are placed on the cell layer in the centre of each of the replicate dishes. Cell cultures are examined through microscope after 24 and 48h of incubation to assess the presence or absence of cytotoxic effects due to the sample.

Target cells: BSCL 56 /L 929 (Mouse connective tissue)

Culture medium: Minimum Essential Medium (MEM) with Earle's salts added with 5 % of foetal bovine serum, 1 % of L-glutamine, 0,6 % of penicillin/streptomycin and 0,3 % of fungizone (complete MEM).

Sample preparation: A portion of the sample that covers approximately one-tenth of the cell layer surface is placed in direct contact with the cell cultures.

Positive control: A portion of latex of natural rubber that covers approximately one-tenth of the cell layer surface.

Negative control: A portion of silicone rubber that covers approximately one tenth of the cell layer surface.

Vehicle control: Complete Cell Culture Medium MEM.

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Incubation: The dishes treated with the test specimen, with the Positive and Negative controls and with the Cell Culture Medium control are incubated for 48 h at 37 ± 1 °C in a 5% CO_2 atmosphere.

Apparatus

Incubator, which maintains the cultures at 37°C, 5% CO_2;
Microscope, with inverted phase contrast optics;
Laminar Flow Cabinet;
Sterile Disposable;
Tissue Culture Dishes.

Interpretation of Results: The determination of the cytotoxicity is performed after a 24 and 48 h incubation period examining the cells under the microscope to assess general morphology, vacuolation, detachment, cell lysis, membrane integrity.
The change from normal morphology of the Negative control is rated on a reactivity grade from 0 to 4 (see Grading system). Moreover, for the dishes treated with the Test specimen the confluence of the monolayer is evaluated and the color of test medium is compared to the negative control

Grading system:

Grade	Reactivity	Description of Reactivity Zone
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

Considering the employed test method in performing cytotoxicity test and the sample characteristics, eventual absence of cells under the negative control and the specimen should be ascribed to physical or mechanical trauma and not to a cytotoxic effect of the negative control and of the sample. Consequently a cytotoxic score is assigned exclusively evaluating the cells around the specimen.

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Results after 24 h incubation	**Score**
Positive control	4
Positive control	4
Positive control	4
Negative control	0
Negative control	0
Negative control	0
MEM control	0
MEM control	0
MEM control	0
Test specimen	0
Test specimen	0
Test specimen	0
Confluency of the monolayer	Confluent
Color of test medium	Comparable to the negative control

Results after 48 h incubation	**Score**
Positive control	4
Positive control	4
Positive control	4
Negative control	0
Negative control	0
Negative control	0
MEM control	0
MEM control	0
MEM control	0
Test specimen	0
Test specimen	0
Test specimen	0
Confluency of the monolayer	Confluent
Color of test medium	Comparable to the negative control

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OPINIONS AND INTERPRETATIONS – Not included in ACCREDIA accreditation

The cells treated with the Test sample after 24 and 48 hours of incubation do not show any changes from normal morphology of the Negative control. The Test sample does not show any reactivity *(grade = 0)*.

The present test report exclusively refers to the referenced test sample.
If the sample has been sampled by the Customer, the results are referred to the sample as received.
The present test report may not be partially reproduced without Biochem authorization.

(#) Data provided by the Customer. The laboratory declines responsibility for such data.

The present test report replaces test report n°20- 0754-01
Reason of the amendment: missing indication of cytotoxicity grade in “Opinions and interpretations” paragraph. Ref. e-mail on info@biochem-bcm.com of 09/07/2020.

Test verified by: Buriani Giampaolo, PhD.

Issue authorized by:
Test Director Giovanni Bassini, Ch.Eng.

Zola Predosa, 10/07/2020

END OF TEST REPORT

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