TESTS FOR IN VITRO CYTOTOXICITY

Test Substance

Hemo Treat Ointment Camphor, Calcium carbonate, Lanolin, White Petrolatum, Adeps Suillus, Eucalyptus oil, Ethanol, Preservative

Test Report N°1572-1/14

Test performed for

Messrs.
SC Global Treat SRL
157, Portului street - Administrative Building, Room 222
6200 GALATI - ROMANIA

by

BIOCHEM S.a.s.
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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 the 14/11/2014 and was completed on the 20/11/2014.

LAB N. 0283
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Ref. Your Order N.7 of the 10/11/2014

Sample description

Description: Hemo Treat Ointment Camphor, Calcium carbonate, Lanolin, White Petrolatum, Adeps Suillus, Eucalyptus oil, Ethanol, Preservative
Code: HT50
Lot: 50HT003
Sterilization: NO
Receipt number: 1572-1
Receipt date: 10/11/2014
Sampling carried out by: SC Global Treat SRL

Note: the test has been performed according to the Cytotoxicity Test Protocol N°118/14.

Test Method

ISO 10993-5: 2009

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, for the Positive control and for the Extraction solvent control. In the dishes to be treated with the sample, the medium is aspirated and replaced with test extract. Cell cultures are examined microscopically after 24 and 48h-contact to assess the presence or absence of cytotoxic effects due to the test extract.

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

Extraction conditions: 0,2 grams of the sample were extracted with 1 ml of Cell Culture Medium MEM with serum at 37°C for 72 hours. The extract was tested undiluted, 1:10 and 1:50 diluted.

Reagents: MEM with Earle's salts added with foetal bovine serum, L-glutamine and some antibiotics.

Positive control: Latex.

Negative control: 4 g of polycarbonate extracted as the sample.

Incubation: The dishes treated with the Test extract, with the Positive and Negative controls and with the Extraction solvent control are incubated for 48 h at 37 ± 1 °C in a 5% CO_2 atmosphere.

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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 extract the confluence of the monolayer is evaluated and the color of test medium is compared to the negative control

Grading system

Grade	Reactivity	Reactivity description
0	None	Discrete intracytoplasmic granules; no cell lysis.
1	Slight	Not more than 20% of the cells are round, loosely attached and without intracytoplasmic granules; occasional lysed cells are present
2	Mild	Not more than 50% of the cells are round and devoid of intracytoplasmic granules; no extensive cell lysis and empty areas between cells
3	Moderate	Not more than 70% of the cell layers contain rounded cells or are lysed
4	Severe	Nearly complete destruction of the cell layers

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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
Extract undiluted	2
Extract undiluted	2
Extract undiluted	2
Confluency of the monolayer	Not confluent
Color of test medium	Comparable to the negative control
Extract diluted 1:10	0
Extract diluted 1:10	0
Extract diluted 1:10	0
Confluency of the monolayer	Confluent
Color of test medium	Comparable to the negative control
Extract diluted 1:50	0
Extract diluted 1:50	0
Extract diluted 1:50	0
Confluency of the monolayer	Confluent
Color of test medium	Comparable to the negative control

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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
Extract undiluted	2
Extract undiluted	2
Extract undiluted	2
Confluency of the monolayer	Not confluent
Color of test medium	Comparable to the negative control
Extract diluted 1:10	0
Extract diluted 1:10	0
Extract diluted 1:10	0
Confluency of the monolayer	Confluent
Color of test medium	Comparable to the negative control
Extract diluted 1:50	0
Extract diluted 1:50	0
Extract diluted 1:50	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 undiluted test extract after 24 hours and 48 hours of incubation show some changes from normal morphology of the Negative control. The undiluted test extract show a mild reactivity after 24 hours and 48 hours of incubation. The cells treated with the 1:10 and 1:50 diluted test extracts after 24 hours and 48 hours of incubation do not show any changes from normal morphology of the Negative control.

Test carried out by: Guardiani Silvia, Dr.

Test verified by: Bongiovanni Lorena, Dr.

Issue authorized by:
Test Director Giovanni Bassini, Ch.Eng. (Signature may be at the bottom)

Zola Predosa, 24/11/2014

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