SIRIM Berhad
Industrial Biotechnology Research Centre,
Tel: 03-55446953/6960 Fax:03-55446988

TEST REPORT

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Applicant	:	Lifeways Sdn Bhd 28, Jalan Jasmin 3, Taman Botanic, 41200 Klang, Selangor Darul Ehsan.
Manufacturer / Company	:	-
Test item	:	Syringe (ISR 100108)
Reference standard / Method of Test	:	ISO 10993-5: 2009. Biological evaluation of medical devices. Part 5: Tests for *in vitro* cytotoxicity.
Description of Test item	:	Received test item with the following identification: 1. Type: Inspiro Syringe Sterile,Non-toxic, Non-pyrogenic, Latex Free 2. Lot No: 110530 3. Code No: ISR 100108 4. Quantity: 3 pieces 5. Volume: 10 cc/mL
Date Received	:	11 June 2012
Job No.	:	J111/12
Issue Date	:	25 JUN 2012

Approved signatories,

(NOOR RABIHAH AID)
Researcher,
Industrial Biotechnology Research Centre,
SIRIM Berhad.

(DR. NEELAM SHAHAB, *AMIC*)
Senior Principal Researcher,
Industrial Biotechnology Research Centre,
SIRIM Berhad.

SIRIM Berhad
(No. Syarikat 367474 - V)
1, Persiaran Dato' Menteri
Seksyen 2, Peti Surat 7035
40911 Shah Alam
MALAYSIA
Tel: 60-3-55446000
Toll free: 1 300 88 7035
Faks: 60-3-55108095
Website : www.sirim.my

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1.0 Test timetable

Receipt of test item:	11 June 2012
Maintenance of cell culture:	15 – 18 June 2012
Extraction procedure:	18 – 19 June 2012
Treatment:	19 – 20 June 2012
End of test:	20 June 2012

2.0 Test method

2.1 Test summary

The degree of cytotoxicity in a mammalian cell culture in response to the test item extracted and diluted in growth medium was determined. Extraction of **Syringe (ISR 100108)** was carried out at (37±1) °C for 24 hours using cell growth medium as extractant. Positive and negative controls were included in the study to verify the proper functioning of the test system. The test item was tested in triplicates at six concentrations: 0.2, 0.1, 0.05, 0.025, 0.012, and 0.006 g/mL. Treatment was carried out at (37±1) °C in a carbon dioxide incubator and assessment carried out after 24-hour incubation.

2.2 Significance and rationale

This method is useful for assessing the cytotoxic potential of new materials and formulations and as part of a quality control program for established medical devices and components. Assessment of cytotoxicity provides useful information in predicting the potential clinical applications in human. Cell culture methods have shown good correlation with animal assays and are frequently more sensitive to cytotoxic agents.

2.3 Cell culture

American Type Culture Collection CCL81, Vero (Kidney, African Green Monkey, *Cercopithecus aethiops*).

2.4 Test procedure

The procedure was divided into three stages as follows.

2.4.1 Cell culture maintenance

Cells were grown in tissue culture grade flasks and routinely examined to ensure they remain healthy. Cells were seeded into 24-well plate and incubated until attaining confluence or near confluence monolayer growth before the treatment procedure.

2.4.2 Preparation of test item

The test item was extracted in the extraction vehicle to give a final extract concentration of approximately 0.2 g/mL. The positive and negative controls included were zinc sulfate and polypropylene, respectively. Growth medium was used as the extraction vehicle. The test item and controls were extracted/prepared in the growth medium and incubated simultaneously at (37±1) °C for 24 hours.

2.4.3 Effect on cell culture

The extract was tested at six concentrations, in growth media: 0.2, 0.1, 0.05, 0.025, 0.012, and 0.006 g/mL. Growth medium from the 24-well plate was replaced with test extracts, in triplicate wells at each concentration. The positive, negative and growth medium controls were included in the study. The plate was incubated for 24 hours at (37±1) °C in a humidified atmosphere of 5 % carbon dioxide and 95 % air.

2.5 Assessment of result

The condition of cultures in each well, before and after treatment, was examined microscopically and graded. The qualitative morphological grading of cytotoxicity is presented in Table 1.

Table 1. Qualitative cytotoxicity grade

Grade	Reactivity	Conditions of cultures
0	None	Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth.
1	Slight	Not more than 20% of the cells are round, loosely attached and without intracytoplasmatic granules, or show changes in morphology; occasional lysed cells are present; only slight growth inhibition observable.
2	Mild	Not more than 50% of the cells are round, devoid of intracytoplasmatic granules, no extensive cell lysis; not more than 50% growth inhibition observable.
3	Moderate	Not more than 70% of the cell layers contain rounded cells or are lysed; cell layers not completely destroyed, but more than 50% growth inhibition observable.
4	Severe	Nearly complete or complete destruction of the cell layers.

3.0 Results

Microscopic examination of cultures before and after treatment with test extracts and controls, each in triplicate wells, are presented in Table 2.

Table 2. Conditions of cultures before and after treatment

Test extracts and controls	Conditions of cultures	
	Before treatment	After treatment
Test extract, 0.2 g/mL	Subconfluent monolayer	Healthy confluent monolayer in all wells
Test extract, 0.1 g/mL	Subconfluent monolayer	Healthy confluent monolayer in all wells
Test extract, 0.05 g/mL	Subconfluent monolayer	Healthy confluent monolayer in all wells
Test extract, 0.025 g/mL	Subconfluent monolayer	Healthy confluent monolayer in all wells
Test extract, 0.012 g/mL	Subconfluent monolayer	Healthy confluent monolayer in all wells
Test extract, 0.006 g/mL	Subconfluent monolayer	Healthy confluent monolayer in all wells
Medium control	Subconfluent monolayer	Healthy confluent monolayer in all wells
Negative control	Subconfluent monolayer	Healthy confluent monolayer in all wells
Positive control	Subconfluent monolayer	Complete destruction of cell layers in all wells with 200 µg/mL zinc sulfate

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4.0 Analysis and interpretation

Conditions of cultures after treatment (from Table 2), each in triplicate wells, was qualitatively graded according to Table 1 and presented in Table 3.

Table 3. Summary of cytotoxic gradings on cultures

Test extracts and controls	Grade	Reactivity
Test extract, 0.2 g/mL	0-0-0	None
Test extract, 0.1 g/mL	0-0-0	None
Test extract, 0.05 g/mL	0-0-0	None
Test extract, 0.025 g/mL	0-0-0	None
Test extract, 0.012 g/mL	0-0-0	None
Test extract, 0.006 g/mL	0-0-0	None
Medium control	0-0-0	None
Negative control	0-0-0	None
Positive control	4-4-4	Severe

5.0 Conclusion

The test item **Syringe (ISR 100108)** exhibited no cytotoxic reactivity at the 0.2 g/mL extract concentrations under the conditions of this test.