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**Testing Center of Radiological
Medical Research Institute,
Soochow University
Test Report**

SpO2 Diaposal Sensor

(Main parts including: inner tape,
top tape, cable, and connector)

Biocompatibility Test

Sample Supplier

Shenzhen Envisen Industry Co.,Ltd.

Supplementary Explanation

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2. Testing report is void without analysis center seal.
3. Valid report must be signed by ones who analyzed, reviewed and issued it.
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5. Testing result of supply sample is only responsible for the provided sample.
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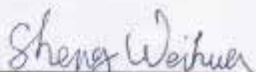
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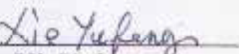
Cytotoxicity Test

Summary

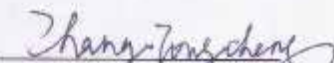
The test article, SpO2 Diaposal Sensor(Main parts including: inner tape, top tape, cable, and connector) was evaluated for cytotoxicity test in accordance with the ISO 10993-Part 5: Tests for cytotoxicity: in vitro methods. The testing sample solution is mixed with growing-well L-929 cell, and then incubated for 2 and 4 days. Observe the morphology and the cell growing on the culture bottle wall. The RGR (Relative Growth Rate) are 0% and 0% respectively by calculation on the basis of cell concentrations of different groups. The RGR of testing group is determined as above Grade 4. This means the testing sample has severe toxicity to L-929 cells.

Tested by:


Sheng Weihua


Xie Yufeng

Checked by:


Zhang Tongcheng

Date completed: Feb 28 , 2006

Introduction

The test article, SpO₂ Diaposal Sensor(Main parts including: inner tape, top tape, cable, and connector) were evaluated for cytotoxicity test in accordance with the ISO 10993-5-1999 Part 5: Tests for cytotoxicity: in vitro methods .The purpose of this study was to determine the potential cytotoxicity of the testing article to L-929 cell.

Materials

1. Test Sample:

1.1 Sample Supplier: ShenZhen Envisen Industry Co.,Ltd.

1.2 Sample Name: SpO₂ Diaposal Sensor(Main parts including: inner tape, top tape, cable, and connector)

1.3 Size: /

1.4 Lot No: /

1.5 Receiving Date: Feb 18, 2006

2. Sample and Control Preparation:

2.1 Testing sample group

The testing sample is treated in accordance with experiment requirements followed by sterilization in autoclave.

24 hours prior to experiment, add liquid culture medium in the proportion of 1ml culture medium to 0.2g testing sample, and then incubated at 37°C for 24 hours. Thus, the sample solution is obtained.

2.2 For the positive controls, add con. 6.3% phenylhydroxide solution.

2.3 For the negative controls, add fresh 1640 liquid culture medium.

3. Culture medium:

RPMI 1640, calf serum, PBS, Pancreatin and double antibiotic.

Test method

1. Cell Strain: Recommended cell lines are American Type Culture Collection CCLI (NCTC clone 929).

2. Cell Culture

2.1 Take growing-well cell of strain L-929 to prepare cell soliquiod with concentration of 4×10^4 counts/ml in culture bottles. Proceed to next step after the cell grows up to monolayer.

2.2 Discard the liquid in culture bottles. In these culture bottles. Take testing group, add con. 50% sample solution, the positive control solution and the negative control solution. They are all incubated at 37°C. After 2 and 4 days incubation respectively, conduct the morphology evaluation and cell counting.

Results

The testing sample solution is mixed with growing-well L-929 cell, and then incubated for 2 and 4 days. Observe the morphology and the cell grow well sticking on the culture bottle wall. The RGR (Relative growth Rate) are 0% and 0% respectively by calculation on the basis of cell concentrations of different groups (the negative and positive controls).

Conclusion

By the experiment incubating L-929 cell using culture medium with sample solution, the RGR of testing group is determined as above Grade 4. This means the testing sample has severe toxicity to L-929 cell.

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Table 1. Classification system for the RGR

Grade	RGR (%)	Reactivity
0	≥ 100	none
1	75-99	slight
2	50-74	moderate
3	25-49	severe

Delayed Contact Sensitization Study (A Maximization Method) In the Guinea Pig

Summary

A guinea pig maximization test (ISO10993-10: 2002) of sample DisposalSpO₂ Sensor(Main parts including: inner tape, top tape, cable, and connector) was conducted to evaluate the potential for delayed dermal contact sensitization .The method of Magnusson and Kligman (1970) was adapted for alcohol in a 0.9% sodium chloride USP solution (AS) test article extract.

The AS extract of the test article was intradermally injected and occusively patched to ten guinea pigs in an attempt to induce sensitization. Following a recovery period, the original ten test and five previously untreated control animals received a challenge patch of the test article extract and the control vehicle .In addition the test article was applied the same animals. All sites were scored at 24, 48, 72 and 96 hours after patch removal.

Under the conditions of this study, the AS test article extract and the test article showed no signification evidence of causing delayed dermal contact sensitization in the guinea pig.

Study and Supervisory Personnel:

Liu Yanbin

Liu Yanbin

Wang Hongyun

Wang Hongyun

Checked by:

Zhang Tongcheng

Zhang Tongcheng

Date completed: Mar 18, 2006

Introduction

A guinea pig maximization test of the material identified below was conducted to evaluate the potential to cause delayed dermal contact sensitization. The test article was received on Feb 18, 2006. The method of Magnusson and Kligman, as reported in Allergic Contact Dermatitis in the Guinea Pig, 1970, was employed with adaptations for a test article extract. The susceptibility of the Hartley guinea pig strain to a known sensitizing agent, 1-chloro-2,4-dinitrobenzene (DNCB), has been substantiated at TCRSU with this method under lab number TCRSU-2002-011 completed on March 28, 2002.

Materials

The sample provide by the sponsor was identified and handled as follows:

Test Article: SpO2 Diaposal Sensor(Main parts including: inner tape, top tape, cable, and connector)

Size: /

Lot No: /

Storage Conditions: Room temperature

Vehicle: alcohol in saline 1:20 solution (AS)

Preparation: For each phase of this test, a ratio of 0.2g: 1ml(test article to volume of vehicle)was used for the test extract . The test article was extracted in AS at 37°C for 24 hours. For the challenge phase ,the vehicle (without test article)was similarly prepared to serve as the control .In addition ,the test article (as received)was cut into 2×2cm sections at the challenge phase .

Condition of Extracts: TEST

CONTROL

Induction I: clear with test article particulates*

Not applicable

Induction II: clear with test article particulates

Not applicable

Challenge: clear with test article particulates

Clear

*Filtered with a 0.8um filter disc to yield a clear particulate free extract

Additional Materials: Freund's Complete Adjuvant (FCA) was used at induction I, and a 10%(w/w)sodium lauryl sulfate (SLS)suspension in petrolatum was used for induction II.These materials were provided by the test facility .

Method

Test System:

Species: Albino Guinea pig Sex: Male

Source: Provided by Animal Center, TCRSU <Permit Code: SCXK(SU)2002-0008>

Body Weight Range: 308 grams to 375 grams at first treatment

Acclimation Period: Minimum 5days

Number of Animals: 15 Identification Method: Ear punch

Justification of Test System:

The albino guinea pig has been used historically for sensitization studies (Magnusson and Kligman ,1970) . The guinea pig is believed to be the most sensitive animal model for this type of study .the susceptibility of the guinea pig to a known sensitizing agent, 1-chloro-2,4-dinitrobenzene (DNCB) has been substantiated at TCRSU with this method .

Animal Management:

Husbandry : Refer to ISO 10993-10-2002 Annex c: Animal and husbandry.

Food: All-nutrient animal food Provided by Suzhou (Twin-lion) Experimental

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- Animal Food Science & Technology Service Co .Ltd.
- Water: Drinking water met the sanitary standard
- Housing: Animals were housed in groups in stainless steel suspended cages identified by a card indicating the lab number , animal numbers, test code , sex, animal code and first treatment date .
- Environmental: The room temperature was monitored daily .The recommended temperature range for the guinea pig was 20-25°C.
The room humidity was monitored daily .The recommended humidity range for the guinea pig was 30-70%.
- Personnel: Associates involved were appropriately qualified and trained.
- Selection: Only healthy, previously unused animals were selected .

Induction I:

The hair was removed with an electric clipper from an area of the back over the dorsoscapular region of ten guinea pigs designated as test animals. Three rows of intradermal injections (two per row) were given to each animal within an approximate 2cm×4cm boundary of the fur clipped area as illustrated below :

4cm		
A	A	2cm
B	B	
C	C	

- 0.1ml of FCA
- 0.1ml of AS test article extract
- 0.1ml of 1:1suspension of the AS test article extract and FCA

Five untreated guinea pigs were simply maintained during the induction phases as controls to be used during the challenge phase. The group of animals was not induced so that any background or primary irritant response could be differentiated from true sensitization at the challenge phase.

Induction II:

One week after the injections , the same area used during induction I was reclipped with an electric clipper. The 10% SLS suspension in petrolatum was massaged into the skin over the injection site to provoke a mild acute inflammation .The area was left uncovered .The day after the SLS administration,any remaining SLS residue was gently removed with a gauze pad .A 2cm×4cm section of Whatman No.3MM filter paper ,saturated with 0.3ml of freshly prepared AS test article extract, was then topically applied to the previously injected sites of the test animals .Each patch was secured with a nonreactive tape and the trunk of each animal was wrapped with an elastic bandage .At 48 hours ,the binders and patches were removed.

Challenge:

At 13days after the induction patch , the hair of each test and previously untreated control guinea pig was clipped over the flank areas as needed .For each animal, the contained in a chamber was saturated with 0.3ml of the AS test article extract or control vehicle.In addition ,a 2cm×2cm patch of the test article (as received)was prepared for each animal .All patches were topically applied as indicated below:

TREATMENT GROUP(n)	CHALLENGE SITE	
	LEFT FLANK	RIGHT FLANK (SITE)
Test(10)	Control Vehicle	Test Article(upper) Test Extract(lower)
Control(5)	Control Vehicle	Test Article(upper) Test Extract(lower)

Each patch was secured to the skin with semiocclusive hypoallergenic adhesive tape. The trunk of each animal was wrapped with an elastic bandage to maintain well-occluded sites for the 24 hour exposure.

Observations for dermal reactions were conducted at 24,48,72,and 96 hours after challenge patch removal . The sites were wiped gently with gauze after patch removal .Prior to scoring at each interval ,sites were wiped with 35%isopropyl alcohol .Scores were recorded in accordance with the criteria shown below:

SCORE	OBSERVATION
0	No visible reaction
0.5	Very faint erythema usually nonconfluent
1	Faint erythema usually confluent
2	Moderate erythema
3	Strong erythema with or without edema

The response , pattern, character, and duration of any test animal reactions were compared to any reactions in the control conditions .Any dermal inflammatory response at the test sites greater than that seen in any control condition was considered evidence of a potential allergic response .Background or artifactual reactions(0.5 score) were not counted as evidence of a sensitization response.

Based on the number of guinea pigs considered as sensitized, the allergenicity rating was assessed as follows:

%Reacting Animals	Testing	Classification
0%		Not a Sensitizer
1~10%		Weak Sensitizer
11~30%		Mild Sensitizer
31~60%		Moderate Sensitizer
61~80%		Strong Sensitizer
81~100%		Extreme Sensitizer

Note: In the Magnusson and Kligman model, weak sensitization is not regarded as significant.

Result

Individual results of dermal scoring for the challenge appear in Table 1. Only in significant background reactions (scores of 0.5) were noted. No evidence of sensitization was observed.

Clinical Observations: All animals appeared clinically normal throughout the study.

Results and conclusions apply only to the test article tested. No further evaluation of these results is made by TCRSU. Any extrapolation of these data to other samples is the responsibility of the sponsor.

Conclusion

Under the conditions of this study, the as test article extract and the test article showed no significant evidence of causing delayed dermal contact sensitization in the guinea pig.

**Table 1 GUINEA PIG SENSITIZATION
DERMAL REACTIONS – CHALLENGE**

Animal Number/ Group	HOURS FOLLOWING PATCH REMOVAL											
	24			48			72			96		
	SITE	SITE	SITE	SITE	SITE	SITE	SITE	SITE	SITE	SITE	SITE	SITE
	A	B	C*	A	B	C*	A	B	C*	A	B	C*
1 Test	0	0	0	0	0	0	0	0	0	0	0	0
2 Test	0	0	0	0	0	0	0	0	0	0	0	0
3 Test	0	0	0	0	0	0	0	0	0	0	0	0
4 Test	0	0	0	0	0	0	0	0	0	0	0	0
5 Test	0	0	0	0	0	0	0	0	0	0	0	0
6 Test	0	0	0	0	0	0	0	0	0	0	0	0
7 Test	0	0	0	0	0	0	0	0	0	0	0	0
8 Test	0	0	0	0	0	0	0	0	0	0	0	0
9 Test	0	0	0	0	0	0	0	0	0	0	0	0
10 Test	0	0	0	0	0	0	0	0	0	0	0	0
11 control	0	0	0	0	0	0	0	0	0	0	0	0
12 control	0	0	0	0	0	0	0	0	0	0	0	0
13 control	0	0	0	0	0	0	0	0	0	0	0	0
14 control	0	0	0	0	0	0	0	0	0	0	0	0
15 control	0	0	0	0	0	0	0	0	0	0	0	0

Site A = Left Flank = AS control vehicle

Site B = Lower Right Flank = AS test extract

Site C = Upper Right Flank = 2 × 2cm patch of test article

*-mechanical trauma and/or hair loss due to the adhesive nature of the test article noted on each site

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Skin Irritation Test Summary

The test article, SpO2 Diaposal Sensor(Main parts including: inner tape, top tape, cable, and connector), was evaluated for primary skin irritation in accordance with the ISO 10993-Part 10-2002: Tests for Irritation and Sensitization. Observe and write down the skin responses on injection sites in 24, 48 and 72 hours respectively after injection. The skin responses include erythema and oedema. Grade the tissue reaction for erythema and oedema according to the classification system given in Table 1. According to what observed, the response of skin on testing side does not exceed that on the control side. The primary irritation index for the test article was calculated to be 0. The test result shows that leached solution of sample does not induce irritation to rabbit skin.

Study and Supervisory Personnel:

Mi Zhisu
Mi Zhisu

Wang Hongyun
Wang Hongyun

Checked by:

Zhang Tongcheng
Zhang Tongcheng

Date completed: Feb 25, 2006

Signed by:

Zhang Tongcheng


Introduction

The test article, SpO₂ Diaposal Sensor(Main parts including: inner tape, top tape, cable, and connector), was evaluated for primary skin irritation in accordance with the guidelines of the ISO 10993.10—2002 Part 10: Tests for irritation and sensitization. This study was to determine the potential skin irritation after the injection of sample solution into the animal back. The test article was received on Feb 18, 2006. Injections were applied on Feb 21, 2006.and the observations were concluded on Feb 24, 2006.

Materials

Test Article: DisposalSpO₂ Sensor(Main parts including: inner tape, top tape, cable, and connector)

Size: /

Lot No: /

Storage Conditions: Room temperature

Sample and Control Preparation

The samples are rinsed with redistilled water and blotted up with filter paper. In a container with cut sample, add the leaching solution (0.9% NaCl injection solution) in the proportion of 1ml leaching solution to 0.2g sample. Seal up the container at 37°C for 24 hours. The solution so prepared is termed as leached solution of sample. The control solution (0.9% NaCl) is obtained by same way but absent of sample.

Method

Test System:

Species: Three rabbits.

Breed: New Zealand white (single strain)

Source: Provided by Animal Center, TCRSU<Permit Code: SCXK (SU)2002-0008>

Sex: Female.

Body Weight Range: 1.8-2.0 kg at patching.

Age: Young adult

Acclimation Period: Minimum 5 days.

Number of Animals: Three

Identification Method: Ear tag

Justification of Test System:

The rabbit is specified as an appropriate animal model for evaluating potential skin irritants by the current ISO testing standards. The rabbit is widely used for this purpose and relative ranking of irritant scores can be determined.

Animal Management:

Husbandry: Refer to ISO 10993-10: 2002 Annex C: Animal and husbandry

Food: All-nutrient animal food provided by Suzhou (Twin-lion) Experimental Animal Food Science & Technology service Co., Ltd.

Water: Provided by Sanitary Standard for drinking water.

Housing: Animals were individually housed in stainless steel suspended cages identified by a card indicating the lab number, animal number, test code, sex, and date dosed.

Environmental: The room temperature was monitored daily. The temperature range for the rabbit was 20-25°C. The room humidity was monitored daily. The humidity range for the rabbit was 30-70%.

Personnel: Associates involved were appropriately qualified and trained.

Selection: Only healthy, previously unused, animals free from irritation or other dermatological lesions that could interfere with the test were selected.

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Experimental Procedure

Clean the rabbit's naked skin with 75% alcohol. Choose ten points at 2cm intervals on one side of rabbit back and inject 0.2ml leached solution at each point. Similarly, on the other side of rabbit back. Choose five points at 2cm intervals and inject 0.2ml control solution at each point.

Observe and write down the skin responses of injection sites in 24h, 48h and 72h respectively after injection. The skin response include erythema, odema and necrosis as well. From weak to serious, the responses are differentiated by grade 0, 1, 2, 3, 4 on the basis of its extent. See table 1.

Result

According to what observed, the response of skin on testing side does not exceed that on the control side. Thus, it is identified as grade 0. See table 2.

Conclusion

The test result shows that leached solution of sample does not induce irritation to skin.

Record Storage

All raw data pertaining to this study and a copy of the final report are to be retained in designated SUFY archive files.

TCRSU was awarded Metrology Accreditation Certificate (No. S0174) by National Technology Supervision Bureau in Nov 1, 2004.

Table.1 Classification System for Skin Reaction

<u>Reaction</u>	<u>Numerical Grading</u>
<u>Erythema and Eschar Formation:</u>	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate erythema	3
Severe erythema (beet redness) to eschar formation preventing grading of erythema	4
<u>Edema Formation:</u>	
No edema	0
Very slight edema (barely perceptible)	1
Well-defined edema (edges of area well-defined by definite raising)	2
Moderate edema (raised approximately 1mm)	3
Severe edema (raised more than 1mm and extending beyond exposure area)	4
Total possible score for irritation	8

Irritation Response Categories in the Rabbit

<u>Response Category</u>	<u>Mean score</u>
Negligible	0 to 0.4
Slight	0.5 to 1.9
Moderate	2 to 4.9
Severe	5 to 8

NOTE: Other adverse changes at the skin sites shall be recorded and reported

Table 2. Dermal Observations

Rabbit No			Interval		(hours)
			24	48	72
1	Test	Erythema	0	0	0
		Edema	0	0	0
	Control	Erythema	0	0	0
		Edema	0	0	0
2	Test	Erythema	0	0	0
		Edema	0	0	0
	Control	Erythema	0	0	0
		Edema	0	0	0
3	Test	Erythema	0	0	0
		Edema	0	0	0
	Control	Erythema	0	0	0
		Edema	0	0	0