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TESTING
CNAS L2954

Testing Center of Sanitation & Environment technic Institute, Soochow University, Test Report

Report Number: SDWH-2009-21326

Sample Name: Tape For Wrap Sensor

Testing Item: Biocompatibility Test

Sample Supplier: Shenzhen Envisen Industry
Co., Limited

Testing Center of Sanitation & Environment technic Institute, Soochow University

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Testing Center of Sanitation & Environment technic Institute**Soochow University****Test Report**

Sample name	Tape For Wrap Sensor
Sample supplier	Shenzhen Envisen Industry Co.,Limited
Receiving date	Dec 14, 2009
Size	White/ Blue
Lot No	WM09072901/WM09072902
Testing items	Cytotoxicity Test
	Delayed Contact Sensitization Study in the Guinea Pig
	Skin irritation Test

Edited: MulishenChecked: Pang JingyiApproved: Zhang Tongcheng

Testing Center of Sanitation & Environment technic Institute, Soochow University

Date: Jan 26, 2010

Cytotoxicity Test

Summary

The test article Tape For Wrap Sensor, were evaluated for cytotoxicity test in accordance with the ISO 10993-5:2009(E): Tests for in vitro cytotoxicity The testing sample solution was mixed with growing-well L-929 cell, and then incubated for 24h at 37°C in 5% CO₂. Intracytoplasmic granulation and cell lysis were observed, MTT method was used to determine the potential cytotoxicity The results showed that the cytotoxicity ratio of the 100 % extract of the test sample were 79.8%, the 50 % extract of the test sample were 81.4% the negative control were 100.1%, the positive control cytotoxicity ratio were 1.5%, This meant that the test was valid and the sample article had no toxicity to L-929 cell.

Date completed: Dec 27, 2009

Tester: SU Xiao-jun NING Ping

Introduction

The test article were evaluated for cytotoxicity in accordance with ISO 10993-5:2009(E): Tests for in vitro cytotoxicity. The purpose of this study was to determine the potential cytotoxicity of the testing article to L-929 cell. The study was conducted in accordance with Good Laboratory Practice Regulation USA 21 CFR Part 58.

Test system and test system Management

L-929 mammalian fibroblast cell will be grown in MEM medium with 10% FCS, Cells will be seeded into the 96-well cell culture plates, and incubated at 37°C in a humidified incubator with 5% CO₂ to obtain confluent monolayer of cells prior to use. Aseptic procedures will be used in the handling of cell culture.

Personnel: Associates involved were appropriately qualified and trained.

Justification for selection of the test system

Mammalian cell culture monolayer, L-929 mouse fibroblast cells (American Type Culture Collection CCLI (NCTC clone 929), will be used. In vitro mammalian cell culture study has been used to historically evaluate the cytotoxicity of biomaterial of medical device.

Materials

1. Equipments:

Autoclaves (SDWH-048),
CO₂ Incubator (SDWH-021),
Inverted microscope (SDWH-037),
Super clean working desk (SDWH-047),
Refrigerator (SDWH-448),
Power Wave XS Microplate Reader (SDWH-312),etc.

2. Reagents:

Phenol (Lot No: 20080401),
MTT (Lot No: 13241337),
FCS (Lot No: 743794),
Trypsinase (Lot No: 608501),
MEM (Lot No: 1402389),
Penicilline、Streptomycin sulfate (Lot No: 577993),
Isopropanol (Lot No: 20081112),etc.

3. Cell Strain:

Recommended cell lines are American Type Culture Collection CCLI (NCTC clone 929).

4. Control Preparation:

Negative control: High density polyethylene +MEM medium, with addition 10% FCS. (37°C 24h)

Positive control: MEM medium, with addition 10% FCS and 0.5 % phenol (37°C 24h) .

Blank control: MEM medium, with addition 10% FCS. (37°C 24h)

Test method

1. Cell Strain: Recommended cell lines are American Type Culture Collection CCLI (NCTC clone 929).
2. Preparation of sample Extracts

Sample name	Extraction ratio	Actual sampling	Extraction conditions
white	6cm ² :1ml	36cm ² :6ml	37°C,24 小时
blue	3cm ² :1ml	18cm ² :6ml	37°C,24 小时

Mixing well 2ml extract from each of the tube above as the final extract.

3. Cell culture

L929 cells were cultured in MEM medium, supplemented with 10% FCS at 37°C in a humidified atmosphere of 5% CO₂. L929 cells were digested by using 0.5% trypsin (EDTA) and the single-cell suspension formed 1×10⁵ cells/ml suspended cells were cultured in 96-well plates with 100μl per well. After the cells grew into monolayer, discard the original culture medium, and then add 100μl the sample extraction , positive control solution and negative control solution to each well respectively.

4. Cell morphological observation and evaluation of cytotoxicity

After 24h incubation respectively, take out a 96-well plate for cell morphological observation firstly, and then add 50μl MTT (1mg/mL) to each well. The cells were further cultured for 2 hours when all liquid in each well was tipped out and 100 μl isopropanol was added to dissolve the precipitate. Sample group were Tester dual-wavelength spectrophotometric measurement with the measurement wavelength at 570 nm and reference wavelength at 650 nm. The blank control, negative control and positive control which obtained by same way were tested as the method above.

The cell cytotoxicity ratio of the negative control group, positive control group and sample group are all determined by the following formula:

The cell cytotoxicity ratio = $\frac{[\text{OD}_{570} - \text{OD}_{650}] \text{ of sample group (or } [\text{OD}_{570} - \text{OD}_{650}] \text{ of positive control group)}}{[\text{OD}_{570} - \text{OD}_{650}] \text{ of blank control group}} \times 100\%$

Results

Results in this experiment are showed Table.

Table: Descriptive statistics of the cell vitality

Group	OD570±S	Viab.%
blank	0.993±0.023	100%
Negative control	0.994±0.021	100.1%
positive control	0.015±0.001	1.5%
100% extract of the test sample	0.793±0.017	79.8%
75% extract of the test sample	0.795±0.039	80.1%
50% extract of the test sample	0.808±0.026	81.4%
25% extract of the test sample	0.810±0.006	81.6%

According to ISO10993-5:2009(E) , if viability is reduced to < 70 % of the blank, it has a cytotoxic potential. The 50 % extract of the test sample should have at least the same or a higher viability than the 100 % extract; otherwise the test should be repeated.

The testing sample solution is mixed with growing-well L-929 cell, and then incubated for 24h. The MTT method was used to measure the cytotoxicity ratio , the 100 % extract of the test sample was 79.8%.

Conclusion

The results showed the testing sample had no toxicity to L-929 cell.

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

Summary

A guinea pig maximization test (ISO 10993-10:2002/Amd.1:2006) of sample Tape For Wrap Sensor was conducted to evaluate the potential for delayed dermal contact sensitization. The method of Magnusson and Kligman (1970) was adapted for 0.9% sodium chloride solution test article extract.

The extract of the test article was intradermally injected and occlusively 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 to the same animals. All sites were scored at 24h and 48h after patch removal.

Under the conditions of this study, the test article extract and the test article showed no signification evidence of causing delayed dermal contact sensitization in the guinea pig. (The average dermal scoring for the Positive controls was 2.5, see SDWH-2009-20805)

Date completed: Jan 22, 2010

Tester: LIU Yan-bing, LIANG Yu

Introduction

A guinea pig maximization test of the material identified below was conducted to evaluate the potential to cause delayed dermal contact sensitization. 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 SDWH with this method under lab number SDWH-2009-20805 completed on Sep 25, 2009.

The study was conducted in accordance with Good Laboratory Practice Regulation USA21 CFR Part 58.

Materials

Sample preparation:

Sample and Control Preparation:

Sample name	Extraction ratio	Extraction conditions
white	6cm ² :1ml	70°C, 24 小时
blue	3cm ² :1ml	70°C, 24 小时

Mixing well 2ml extract from each of the tube above as the final extract.

Equipments:

Electric-heated thermostatic water bath (SDWH-418) ,

Autoclave (SDWH-327)

Electronic balance (SDWH-426)

Electronic scale (SDWH-442)

Regents:

0.9%NS

Freund's complete adjuvant (FCA)

10% sodium dodecyl sulfate(10%SDS)

Method

Test System:

Species: Albino Guinea pig

Source: Provided by Animal Center, SDWH <Permit Code: SCXK (SU) 2007-2007>

Acclimation Period: Minimum 5 days

Number of Animals: 15

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 SDWH with this method.

Animal Management:

Husbandry:

Refer to ISO 10993-10:2002/Amd.1:2006: (Animals and husbandry).

Food:

All-nutrient animal foods were provided by Suzhou (Twin-lion) Experimental 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.

Personnel:

Associates involved were appropriately qualified and trained.

Selection:

Only healthy, previously unused animals were selected.

Intradermal induction phase I :

A pair of 0.1ml intradermal injections was made for each of the following, into each animal, at the injection sites (A, B and C) as shown in Figure 1 in the clipped intrascapular region. (If the extract of the test article is unclear, filtering with a 0.8 μ m filter disc to yield a clear particulate free extract)

Site A: A 50:50 (volume ratio) stable emulsion of Freund's complete adjuvant mixed with the chosen solvent.

Site B: The test sample (undiluted extract); the control animals were injected with the solvent alone.

Site C: The test sample at the concentration used at site B, emulsified in a 50:50 volume ratio stable emulsion of Freund's complete adjuvant and the solvent (50%) was injected into the control animals with an emulsion of the blank liquid with adjuvant.

Topical induction phase II :

Seven days (± 1 day) after completion of the intradermal induction phase, the test sample was administered by topical application to the intrascapular region of each animal, using a patch of area approximately 8cm² (filter paper or absorbent gauze), so as to cover the intradermal injection sites. The concentration selected in Intradermal induction phase I for Site B was used. The maximum concentration that could be achieved in Intradermal induction phase I did not produce irritation, when the area was pretreated with 10% sodium dodecyl sulfate massaged into the skin 24h \pm 2h before the patch was applied. The patches were secured with an occlusive dressing. The dressings and patches were removed after 48h \pm 2h.

The control animals were treated similarly, using the blank liquid alone.

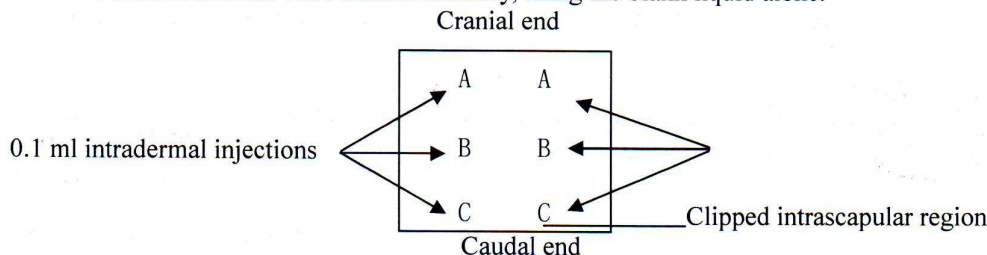


Figure 1-Location of intradermal injection sites

Challenge phase:

At 14 days (± 1 day) after completion of the topical induction phase, all test and control animals were

challenged with the test sample. The test sample and a vehicle control were administered by topical application to sites that were not treated during the induction stage, such as the upper flank of each animal, using appropriate patches or chambers soaked in the test sample at the concentration selected in the intradermal induction phase I for site C. Dilutions of this concentration were also applied to other untreated sites in a similar manner. Occlusive dressings were used to secure areas treated. The dressings and patches were removed after 24 ± 2 h.

Observation of animal:

The appearance of the challenge skin sites of the test and control animal were observed after 24h and 48h removal of the dressings. Natural or full-spectrum was used to visualize the skin reactions. The skin reactions for erythema and oedema were observed and graded according to the Magnusson and Kligman grading given in Table 1 for each challenge site and at each time interval. It is highly recommended that reading be done without knowledge of the treatment, in order to minimize bias in the evaluation of the results.

Evaluation of results:

Magnusson and Kligman grades of 1 or greater in the test group generally indicate sensitization, provided grades of less than 1 are seen in control animal. If grades of 1 or greater are noted in control animal, then the reactions of test animal which exceed the most severe reaction in control animals are presumed to be due to sensitization. If the response is equivocal, re-challenge is recommended to confirm the results from the first challenge. The outcome of the test is presented as the frequency of positive challenge results in the test and control animal.

Table 1 Magnusson and Kligman scale

Patch test reaction	Grading scale
No visible change	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and swelling	3

Result

Individual results of dermal scoring for the challenge appear in Table 2. No evidence of sensitization was observed.

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

Table 2 Guinea pig Sensitization Dermal Reactions – Challenge

Animal Number/ Group	Topical induction phase II	Hours following patch removal (h)		Weight (g)	
		24	48	Before injection	After experiment
1 Test	0	0	0	311	365
2 Test	0	0	0	311	364
3 Test	0	0	0	316	369
4 Test	0	0	0	308	362
5 Test	0	0	0	314	367
6 Test	0	0	0	315	367
7 Test	0	0	0	316	368
8 Test	0	0	0	319	372
9 Test	0	0	0	314	365
10Test	0	0	0	307	361
11control	0	0	0	312	365
12control	0	0	0	310	364
13control	0	0	0	314	368
14control	0	0	0	310	364
15control	0	0	0	313	365

Conclusion

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

Skin irritation Test

Summary

The test article, Tape For Wrap Sensor was evaluated for skin irritation in accordance with the ISO 10993-10:2002/Amd.1:2006: Tests for Irritation and delayed-type hypersensitivity. The skin responses on application sites in 1h, 24h, 48h and 72h respectively after removal the patches (about 2.5cm×2.5cm) which moistened by the extract were observed and recorded. The tissue reaction was graded for erythema and oedema according to the classification system given in Table 1. According to what was 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 positive irritation index was 1.56, see SDWH-2009-21208). The test result showed that the applied sample does not induce irritation to rabbit skin.

Date completed: Jan 8, 2010

Tester: FANG Jing-yi, LIU Chun-li

Introduction

The test article was evaluated for skin irritation in accordance with the guidelines of the ISO 10993-10:2002/Amd.1:2006: Tests for Irritation and delayed-type hypersensitivity. This study was to determine the potential skin irritation after the patches which moistened by the extract apply to the animal back. The test started on Jan 4, 2010, and concluded on Jan 8, 2010.

The study was conducted in accordance with Good Laboratory Practice Regulation USA 21 CFR Part 58.

Materials

Equipments:

Electric-heated thermostatic water bath (SDWH-418) ,Autoclave (SDWH-327)

Electronic balance (SDWH-426) ,Electronic scale (SDWH-442)

Regents: 0.9%NS

Positive control: 20% sodium dodecyl sulfate (20%SDS)

Method

Test System:

Species: Rabbits.

Breed: New Zealand white (single strain)

Source: Provided by Animal Center, TCRSU<Permit Code: SCXK (SU) 2007-2007>

Body Weight Range: Not less than 2 kg.

Age: Young adult

Acclimation Period: Minimum 5 days.

Number of Animals: Three

Justification of Test System:

The rabbit is specified as an appropriate animal model for evaluating potential skin irritants by the current ISO 10993-10:2002/Amd.1:2006 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/Amd.1:2006 (Animals and husbandry)

Food: All-nutrient animal food was provided by the 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.

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.

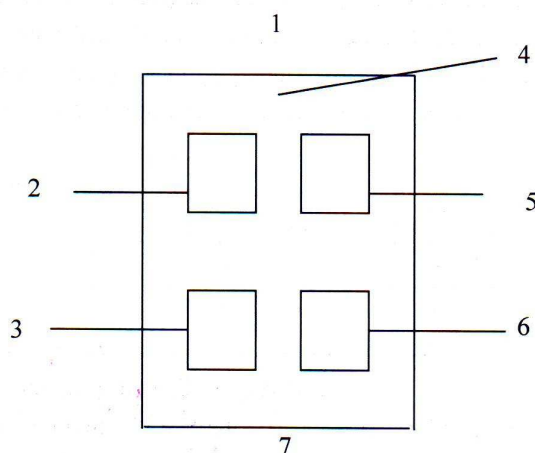
Experimental Procedure

1. Sample and Control Preparation:

Sample name	Extraction ratio	Extraction conditions
white	6cm ² :1ml	70°C, 24 小时
blue	3cm ² :1ml	70°C, 24 小时

Mixing well 2ml extract from each of the tube above as the final extract. Then apply the 0.5ml extract (s) to 2.5cm×2.5cm absorbent gauze patches.

2. Use only animals with healthy intact skin. Fur is generally clipped within 24h to 4h of testing on the backs of the animals a sufficient distance on both sides of the spine for application and observation of all test sites(approximately 10cm×15cm)



1- Cranial end 2- Test site 3- Control site 4- Clipped dorsal region 5- Control site 6- Test site 7- Caudal end

Figure1-Location of skin application sites

Apply the appropriate extract(s) to 2.5cm×2.5cm Absorbant gauze patches. Apply one patch on each side of the animal as shown in Figure 1. Similarly. Apply a control patch of gauze moistened with the extract vehicle as indicated in Figure 1. Then wrap the application sites with a bandage (semi-occlusive or occlusive) for a minimum of 4h. At the end of the contact time, remove the dressing.

Describe and score the skin reaction for erythema and oedema according to the scoring system given in Table 1 and Table 2 for each application site at each time interval. Record the appearance of each application site at 1h, 24h, 48h and 72h following removal of the patches. Use only 24h, 48h, 72h observations for calculation. For each animal add together the primary irritation scores for the test material for both erythema and oedema at each time point and divide the total number of observations.

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.

Table.1 Classification System for Skin Reaction

Erythema and Eschar Formation:	Numerical Grading
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
Edema Formation:	
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	
Response Category	Mean score
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 were recorded and are reported

Table .2 Dermal Observations

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

Conclusion

The test result shows that of the applied 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 SDWH archive files.