180015144061

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TESTING
CNAS L2954

Final Report

Report Number: SDWH-M202003265-4(E)

Skin Irritation Test of Disposable Nitrile Examination Glove

According to ISO 10993-10:2010
0.9% Sodium Chloride Injection Extract

Sponsor: Shanxi Hongjin Plastic Technology Co., Ltd

Address: Coal Bed Gas Industrial Zone, Qu'e Town, Daning County, Linfen City, Shanxi Province

Sanitation & Environment Technology Institute, Soochow University
Address: 199 Ren-Ai Road, Suzhou Industrial Park, Suzhou, Jiangsu 215123, P. R. China
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Content

Supplementary Explanation

(1) Please apply for rechecking within 15 days of receiving the report if there are any objections.

(2) Any erasure or without special inspection and testing seal renders the report null and void.

(3) The report is only valid when signed by the persons who edited, checked and approved it.

(4) The results relate only to the articles tested.

(5) The report shall not be reproduced except in full without the written approval of the institute.

Quality Assurance Statement

The Quality Assurance Unit inspected/audited this study in compliance with the following GLP regulations:

Good Laboratory Practice (GLP) Regulation 21 CFR Part 58, U.S. Food and Drug Administration (FDA). The laboratory is exempt from the following provisions: 21 CFR Part 58.105 Test and Control Article Characterization, and Part 58.113 Mixtures of Articles with Carriers.

The Quality Assurance Unit conducted inspections on the following dates. The findings were reported to the Study Director and to the Testing Facility Management. The final report was reviewed by the Quality Assurance Unit. The final report accurately describes the test methods in accordance with standard operating procedures, and the results are consistent with raw data of non-clinical studies conducted according to the study protocol.

Inspections	Date of Inspection	Date Reported to Study Director	Date Reported to Testing Facility Management.
Study Protocol	2020-06-30	2020-06-30	2020-07-31
Study Procedure	2020-07-03	2020-07-03	2020-07-31
Raw Data	2020-07-31	2020-07-31	2020-07-31
Final Report	2020-07-31	2020-07-31	2020-07-31

Quality Assurance Unit: Zou Jing 2020-07-31

Quality Assurance Date

GLP Compliance Statement

This study was fully in accordance with the technical requirements of the study protocol.

This study was conducted in compliance with Good Laboratory Practice (GLP) Regulation 21 CFR Part 58, U.S. Food and Drug Administration (FDA).

The laboratory is exempt from the following provisions: 21 CFR Part 58.105 Test and Control Article Characterization, and Part 58.113 Mixtures of Articles with Carriers.

Verification Dates

Test Article Receipt	2020-06-23
Protocol Effective Date	2020-06-30
Technical Initiation Date	2020-06-30
Technical Completion Date	2020-07-10
Final Report Completion Date	2020-08-03

Edited by: Chenrongrong 2020-07-31
Date

Reviewed by: 2020-08-03
Study Director Date

Approved by: Fangjingxi 2020-08-03
Authorized Signatory Date

Sanitation & Environment Technology Institute, Soochow University

Summary

1 Test Article

Test Article Name	Disposable Nitrile Examination Glove
Manufacturer	Shanxi Hongjin Plastic Technology Co., Ltd
Address	Coal Bed Gas Industrial Zone, Qu'e Town, Daning County, Linfen City, Shanxi Province
Model	Not supplied by sponsor (N/S)
Lot/Batch	N/S

2 Main Reference

ISO 10993-10:2010 Biological evaluation of medical devices — Part 10: Tests for irritation and skin sensitization

3 Test Method

The extract of test article was evaluated for skin irritation. With ISO 10993-10:2010 Biological evaluation of medical devices — Part 10: Tests for irritation and skin sensitization.
Study protocol number: SDWH-PROTOCOL- GLP-M202003265-4.

4 Conclusion

The test result showed that the response of the test article extract was categorized as negligible under the test condition.

Test Report

1 Purpose

The extract of test article was evaluated for skin irritation and extrapolating the results to humans, but it does not establish the actual risk of irritation.

2 Reference

ISO 10993-10:2010 Biological evaluation of medical devices — Part 10: Tests for irritation and skin sensitization
ISO 10993-12:2012 Biological evaluation of medical devices — Part 12: Sample preparation and reference materials
ISO 10993-2:2006 Biological evaluation of medical devices — Part 2: Animal welfare requirements

3 Compliance

Good Laboratory Practice Regulations, 21 CFR, Part 58.
ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories (CNAS—CL01 Accreditation criteria for the competence of testing and calibration laboratories) China National Accreditation Service for Conformity Assessment LABORATORY ACCREDITATION CERTIFICATE Registration No. CNAS L2954.
RB/T 214—2017 Competence assessment for inspection body and laboratory mandatory approval—General requirements for inspection body and laboratory Certification and Accreditation Administration of the People's Republic of China INSPECTION BODY AND LABORATORY MANDATORY APPROVAL Certificate No. CMA 180015144061.

4 Identification of Test and Control Articles

4.1 Test Article

Test Article Name	Disposable Nitrile Examination Glove
Manufacturer	Shanxi Hongjin Plastic Technology Co., Ltd
Address	Coal Bed Gas Industrial Zone, Qu'e Town, Daning County, Linfen City, Shanxi Province
Test Article Initial State	Not Sterilized
CAS Code	N/S
Model	N/S
Size	M
Lot/Batch	N/S
Test Article Material	nitrile
Packaging Material	N/S
Physical State	pieces
Color	blue
Density	N/S
Stability	N/S
Solubility	N/S
Storage Condition	Room Temperature
Intended Clinical Use	to prevent cross contamination

The information about the test article was supplied by the sponsor wherever applicable.
The Sponsor is responsible for all test article characterization data as specified in the GLP regulations.

4.2 Control Article

4.2.1 Negative Control

Name: 0.9% sodium chloride injection (SC)
Manufacturer: Guangxi Yuyuan Pharmaceutical Co., Ltd.
Size: 500mL
Lot/ Batch#: H20010204
Physical State: Liquid
Color: Colourless
Storage Condition: Room Temperature

4.2.2 Positive Control

Name: sodium dodecyl sulfate
Manufacturer: Ron reagent
Size: 500g
Lot/ Batch#: RH178474
Physical State: Powder
Color: White
Storage Condition: Room Temperature
Solvent: 0.9% sodium chloride injection (SC)
Concentration: 20%
Date prepared: 2020-06-30

5 Equipment and Reagents

5.1 Equipment

Equipment Name	Equipment Number	Calibration Expire
Electronic Scale	SDWH2436	2020-12-10
Horizontal Large Capacity Constant Temperature Vibrator	SDWH2671	2021-01-13
Steel straight scale	SDWH463	2020-07-29
Vertical pressure steam sterilizer	SDWH2097	2021-03-25

5.2 Reagents

Reagent Name	Manufacturer	LOT
0.9% sodium chloride injection (SC)	Guangxi Yuyuan Pharmaceutical Co., Ltd.	H20010204
Sodium dodecyl sulfate (SDS)	Ron reagent	RH178474

6 Identification of Test System

Species: New Zealand white Rabbit (single strain).
Number: 3
Sex: Female
Weigh: Initial body weight not less than 2kg
Health status: Healthy, not previously used in other experimental procedures, young adult, nulliparous and not pregnant.
Housing: Animals were housed in cages identified by a card indicating the lab number, test code and first treatment date.
Animal identification: Stain with dyeing liquid
Cages: Stainless steel cage

Acclimation Period: 7 days under the same conditions as for the actual test

7 Animal Care and Maintenance

Animal purchase: Provided by Suzhou Experimental Animal Sci-tech Co., Ltd. <Permit Code: SCXK (SU) 2015-0007>
Bedding: NA
Feed: Rabbit Diet, Suzhou Experimental Animal Sci-tech Co., Ltd.
Water: Drinking water met the Standards for Drinking Water Quality GB 5749-2006
Animal room temperature: 18-26°C
Animal room relative humidity: 30%-70%
Lights: 12 hours light/dark cycle, full-spectrum lighting
Personnel: Associates involved were appropriately qualified and trained.
Selection: Only healthy, previously unused animals were selected.
There were no known contaminants present in the feed, water expected to interfere with the test data.

8 Justification of Test System and Route of Administration

The rabbit is specified as an appropriate animal model for evaluating potential skin irritants by the current testing standards. Positive control sodium dodecyl sulfate has been substantiated at SDWH with this method. See table 3.
The patches (about 2.5cm×2.5cm) which moistened by test article extract, and directly applying to the rabbit skin is considered to be the best mean of contact.

9 Experimental Design

9.1 Preparation of Extracts

9.1.1 Pretreatment

Autoclaving at 121°C for 30 min.

9.1.2 Extraction

Under aseptic conditions, samples were taken according to the sampling method (Random sampling). The extraction was performed with agitation in closed inert containers according to the extraction ratio listed in the following table (sample: extraction vehicle). The extraction vehicle was SC.

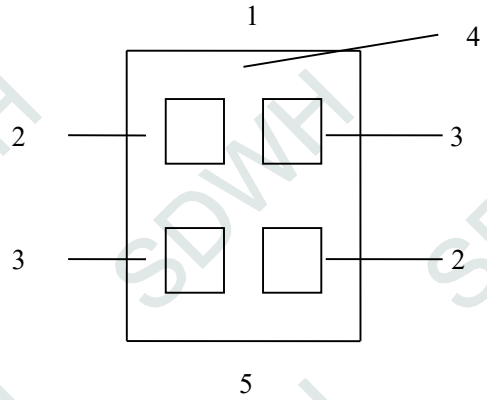
Test Period	Actual Sampling	Extract Procedure			Final Extract
		Extract Ratio	Extraction volume	Condition	
polar test extract	Surface area 120 cm^2	6 cm^2 : 1 mL	20.0 mL	50°C, 72 h	Clear
polar negative control	/	/	10.0 mL	50°C, 72 h	Clear

The state of the extract did not change after extraction. The extract was stored at room temperature, and tested within 24 h, without the process of adjusting its pH value, filtering, centrifuging, diluting, etc. The vehicle (without the test article) was similarly prepared to serve as the control.

9.2 Experimental Procedure

Use the rabbits with healthy intact skin. Fur was generally clipped within 4-24 h of testing on the backs of the rabbits, a sufficient distance on both sides of the spine for application and observation of all test sites (approximately 10cm×15 cm).
Apply 0.5 mL extract (s) of test article or control to 2.5 cm×2.5 cm absorbent gauze patches, and then apply the patch soaked with the extract of test article or control directly to the skin on each side of each rabbit as shown in Figure 1, and then wrap the application sites with a bandage

(semi-occlusive or occlusive) for a minimum of 4 h. At the end of the contact time, remove the dressing and washing with lukewarm water or other suitable nonirritating solvent and careful drying.



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

Figure1 Location of skin application sites

9.3 Observation of Animals

Describe and score the skin reaction for erythema and oedema according to the scoring system given in Table 1 for each application site at each time interval. Record the appearance of each application site at (1±0.1) h, (24±2) h, (48±2) h and (72±2) h following removal of the patches.

Table 1 — Scoring system for skin reaction

Reaction	Irritation score
Erythema and Eschar Formation	
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
Oedema 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
Maximal possible score for irritation	8
Other adverse changes at the skin sites shall be recorded and reported.	

9.4 Evaluation of Results

Use only (24±2) h, (48±2) h and (72±2) h observations for calculation.

After the 72 h grading, all erythema grades plus oedema grades (24±2) h, (48±2) h and (72±2) h were totalled separately for each test sample and blank for each animal. The primary irritation score for an animal was calculated by dividing the sum of all the scores by 6 (two test/observation sites, three time points).

To obtain the primary irritation index for the test article, add all the primary irritation scores of the individual animals and divide by the number of animals.

When blank or negative control is used, calculate the primary irritation score for the controls and subtract that score from the score using the test material to obtain the primary irritation score.

The primary irritation index (PII) for the test article was evaluated according to Table 2.

Table 2 — Primary or cumulative irritation index categories in a rabbit

Mean score	Response category
0~0.4	Negligible
0.5~1.9	Slight
2~4.9	Moderate
5~8	Severe

10 Results

All animals were survived and no abnormal signs were observed during the study. According to what observed, the response of skin on testing side did not exceed that on the control side. Thus, the primary irritation index for the test article was calculated to be 0. See table 4.

11 Conclusion

The test result showed that the response of the test article extract was categorized as negligible under the test condition.

12 Record Storage

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

13 Confidentiality Agreement

Statements of confidentiality were as agreed upon prior to study initiation.

14 Deviation Statement

There were no deviations from the approved study protocol which were judged to have any impact on the validity of the data.

Annex 1 Test Data

Table 3 Positive control

Extract	Rabbit No.	Group	Reaction	Interval (hours): score=left site/right site 24±2h	48±2h	72±2h
SC	1	Positive Control	Erythema	3/3	4/4	4/4
			Oedema	2/2	3/3	3/4
		Negative Control	Erythema	0/0	0/0	0/0
			Oedema	0/0	0/0	0/0
SC	2	Positive Control	Erythema	2/3	3/3	4/4
			Oedema	3/3	3/3	4/3
		Negative Control	Erythema	0/0	0/0	0/0
			Oedema	0/0	0/0	0/0
SC	3	Positive Control	Erythema	3/3	4/3	4/3
			Oedema	3/2	3/3	4/4
		Negative Control	Erythema	0/0	0/0	0/0
			Oedema	0/0	0/0	0/0
The primary irritation score.					6.4	

Note：Positive control performed once every six months, see SDWH-M202003007-1(Completed Date: 2020-07-03).

Table 4 Test Results of Dermal Observations

Extract	Rabbit No.	Group	Reaction	Interval (hours): score=left site/right site 24±2h	48±2h	72±2h
SC	1	Test Article	Erythema	0/0	0/0	0/0
			Oedema	0/0	0/0	0/0
		Negative Control	Erythema	0/0	0/0	0/0
			Oedema	0/0	0/0	0/0
SC	2	Test Article	Erythema	0/0	0/0	0/0
			Oedema	0/0	0/0	0/0
		Negative Control	Erythema	0/0	0/0	0/0
			Oedema	0/0	0/0	0/0
SC	3	Test Article	Erythema	0/0	0/0	0/0
			Oedema	0/0	0/0	0/0
		Negative Control	Erythema	0/0	0/0	0/0
			Oedema	0/0	0/0	0/0
The primary irritation score.					0	

Annex 2 Photograph of Test Article

Annex 3 Information Provided by Sponsor

1 Production Process

Not supplied by sponsor.

2 Other Information

Not supplied by sponsor.

End of Report

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Final Report

Report Number: SDWH-M202003265-5(E)

Skin Irritation Test of Disposable Nitrile Examination Glove

According to ISO 10993-10:2010
Sesame Oil Extract

Sponsor: Shanxi Hongjin Plastic Technology Co., Ltd

Address: Coal Bed Gas Industrial Zone, Qu'e Town, Daning County, Linfen City, Shanxi Province

Sanitation & Environment Technology Institute, Soochow University
Address: 199 Ren-Ai Road, Suzhou Industrial Park, Suzhou, Jiangsu 215123, P. R. China
Website: www.sudatest.com E-mail: med@sudatest.com
Direct: +86 512 65880038 Free: 400 107 8828

Content

Supplementary Explanation

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Quality Assurance Statement

The Quality Assurance Unit inspected/audited this study in compliance with the following GLP regulations:

Good Laboratory Practice (GLP) Regulation 21 CFR Part 58, U.S. Food and Drug Administration (FDA). The laboratory is exempt from the following provisions: 21 CFR Part 58.105 Test and Control Article Characterization, and Part 58.113 Mixtures of Articles with Carriers.

The Quality Assurance Unit conducted inspections on the following dates. The findings were reported to the Study Director and to the Testing Facility Management. The final report was reviewed by the Quality Assurance Unit. The final report accurately describes the test methods in accordance with standard operating procedures, and the results are consistent with raw data of non-clinical studies conducted according to the study protocol.

Inspections	Date of Inspection	Date Reported to Study Director	Date Reported to Testing Facility Management.
Study Protocol	2020-06-30	2020-06-30	2020-07-31
Study Procedure	2020-07-03	2020-07-03	2020-07-31
Raw Data	2020-07-31	2020-07-31	2020-07-31
Final Report	2020-07-31	2020-07-31	2020-07-31

Quality Assurance Unit: Zou Jing 2020-07-31

Quality Assurance Date

GLP Compliance Statement

This study was fully in accordance with the technical requirements of the study protocol.

This study was conducted in compliance with Good Laboratory Practice (GLP) Regulation 21 CFR Part 58, U.S. Food and Drug Administration (FDA).

The laboratory is exempt from the following provisions: 21 CFR Part 58.105 Test and Control Article Characterization, and Part 58.113 Mixtures of Articles with Carriers.

Verification Dates

Test Article Receipt	2020-06-23
Protocol Effective Date	2020-06-30
Technical Initiation Date	2020-06-30
Technical Completion Date	2020-07-10
Final Report Completion Date	2020-08-03

Edited by: Chenrongrong 2020-07-31
Date

Reviewed by: 2020-08-03
Study Director Date

Approved by: Fangjingxi 2020-08-03
Authorized Signatory Date

Sanitation & Environment Technology Institute, Soochow University

Summary

1 Test Article

Test Article Name	Disposable Nitrile Examination Glove
Manufacturer	Shanxi Hongjin Plastic Technology Co., Ltd
Address	Coal Bed Gas Industrial Zone, Qu'e Town, Daning County, Linfen City, Shanxi Province
Model	Not supplied by sponsor (N/S)
Lot/Batch	N/S

2 Main Reference

ISO 10993-10:2010 Biological evaluation of medical devices — Part 10: Tests for irritation and skin sensitization

3 Test Method

The extract of test article was evaluated for skin irritation. With ISO 10993-10:2010 Biological evaluation of medical devices — Part 10: Tests for irritation and skin sensitization.
Study protocol number: SDWH-PROTOCOL- GLP-M202003265-5.

4 Conclusion

The test result showed that the response of the test article extract was categorized as negligible under the test condition.

Test Report

1 Purpose

The extract of test article was evaluated for skin irritation and extrapolating the results to humans, but it does not establish the actual risk of irritation.

2 Reference

ISO 10993-10:2010 Biological evaluation of medical devices — Part 10: Tests for irritation and skin sensitization
ISO 10993-12:2012 Biological evaluation of medical devices — Part 12: Sample preparation and reference materials
ISO 10993-2:2006 Biological evaluation of medical devices — Part 2: Animal welfare requirements

3 Compliance

Good Laboratory Practice Regulations, 21 CFR, Part 58.
ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories (CNAS—CL01 Accreditation criteria for the competence of testing and calibration laboratories) China National Accreditation Service for Conformity Assessment LABORATORY ACCREDITATION CERTIFICATE Registration No. CNAS L2954.
RB/T 214—2017 Competence assessment for inspection body and laboratory mandatory approval—General requirements for inspection body and laboratory Certification and Accreditation Administration of the People's Republic of China INSPECTION BODY AND LABORATORY MANDATORY APPROVAL Certificate No. CMA 180015144061.

4 Identification of Test and Control Articles

4.1 Test Article

Test Article Name	Disposable Nitrile Examination Glove
Manufacturer	Shanxi Hongjin Plastic Technology Co., Ltd
Address	Coal Bed Gas Industrial Zone, Qu'e Town, Daning County, Linfen City, Shanxi Province
Test Article Initial State	Not Sterilized
CAS Code	N/S
Model	N/S
Size	M
Lot/Batch	N/S
Test Article Material	nitrile
Packaging Material	N/S
Physical State	pieces
Color	blue
Density	N/S
Stability	N/S
Solubility	N/S
Storage Condition	Room Temperature
Intended Clinical Use	to prevent cross contamination

The information about the test article was supplied by the sponsor wherever applicable.
The Sponsor is responsible for all test article characterization data as specified in the GLP regulations.

4.2 Control Article

4.2.1 Negative Control

Name: sesame oil (SO)
Manufacturer: Ji'an Qingyuan District luyuanxiangliao. Co. Ltd
Size: 5kg
Lot/ Batch#: 20200312
Physical State: Oily liquid
Color: Pale yellow
Storage Condition: Room Temperature

4.2.2 Positive Control

Name: sodium dodecyl sulfate
Manufacturer: Ron reagent
Size: 500g
Lot/ Batch#: RH178474
Physical State: Powder
Color: White
Storage Condition: Room Temperature
Solvent: Sesame Oil
Concentration: 20%
Date prepared: 2020-06-30

5 Equipment and Reagents

5.1 Equipment

Equipment Name	Equipment Number	Calibration Expire
Electronic Scale	SDWH2436	2020-12-10
Horizontal Large Capacity Constant Temperature Vibrator	SDWH2671	2021-01-13
Steel straight scale	SDWH463	2020-07-29
Vertical pressure steam sterilizer	SDWH2097	2021-03-25

5.2 Reagents

Reagent Name	Manufacturer	LOT
Sesame oil (SO)	Ji'an Qingyuan District luyuanxiangliao. Co. Ltd	20200312
Sodium dodecyl sulfate (SDS)	Ron reagent	RH178474

6 Identification of Test System

Species: New Zealand white Rabbit (single strain).
Number: 3
Sex: Female
Weigh: Initial body weight not less than 2kg
Health status: Healthy, not previously used in other experimental procedures, young adult, nulliparous and not pregnant.
Housing: Animals were housed in cages identified by a card indicating the lab number, test code and first treatment date.
Animal identification: Stain with dyeing liquid
Cages: Stainless steel cage
Acclimation Period: 7 days under the same conditions as for the actual test

7 Animal Care and Maintenance

Animal purchase: Provided by Suzhou Experimental Animal Sci-tech Co., Ltd. <Permit Code: SCXK (SU) 2015-0007>
Bedding: NA
Feed: Rabbit Diet, Suzhou Experimental Animal Sci-tech Co., Ltd.
Water: Drinking water met the Standards for Drinking Water Quality GB 5749-2006
Animal room temperature: 18-26°C
Animal room relative humidity: 30%-70%
Lights: 12 hours light/dark cycle, full-spectrum lighting
Personnel: Associates involved were appropriately qualified and trained.
Selection: Only healthy, previously unused animals were selected.
There were no known contaminants present in the feed, water expected to interfere with the test data.

8 Justification of Test System and Route of Administration

The rabbit is specified as an appropriate animal model for evaluating potential skin irritants by the current testing standards. Positive control sodium dodecyl sulfate has been substantiated at SDWH with this method. See table 3.
The patches (about 2.5cm×2.5cm) which moistened by test article extract, and directly applying to the rabbit skin is considered to be the best mean of contact.

9 Experimental Design

9.1 Preparation of Extracts

9.1.1 Pretreatment

Autoclaving at 121°C for 30 min.

9.1.2 Extraction

Under aseptic conditions, samples were taken according to the sampling method (Random sampling). The extraction was performed with agitation in closed inert containers according to the extraction ratio listed in the following table (sample: extraction vehicle). The extraction vehicle was SO.

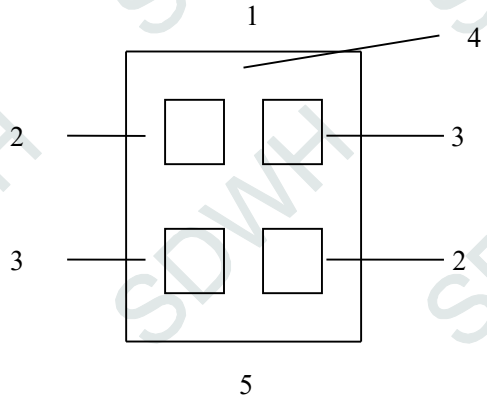
Test Period	Actual Sampling	Extract Procedure			Final Extract
		Extract Ratio	Extraction volume	Condition	
Non-polar test extract	Surface area 120 cm^2	6 cm^2 : 1 mL	20.0 mL	50°C, 72 h	Clear
Non-polar negative control	/	/	10.0 mL	50°C, 72 h	Clear

The state of the extract did not change after extraction. The extract was stored at room temperature, and tested within 24 h, without the process of adjusting its pH value, filtering, centrifuging, diluting, etc. The vehicle (without the test article) was similarly prepared to serve as the control.

9.2 Experimental Procedure

Use the rabbits with healthy intact skin. Fur was generally clipped within 4-24 h of testing on the backs of the rabbits, a sufficient distance on both sides of the spine for application and observation of all test sites (approximately 10cm×15 cm).
Apply 0.5 mL extract (s) of test article or control to 2.5 cm×2.5 cm absorbent gauze patches, and then apply the patch soaked with the extract of test article or control directly to the skin on each side of each rabbit as shown in Figure 1, and then wrap the application sites with a bandage (semi-occlusive or occlusive) for a minimum of 4 h. At the end of the contact time, remove the

dressing and washing with lukewarm water or other suitable nonirritating solvent and careful drying.



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

Figure1 Location of skin application sites

9.3 Observation of Animals

Describe and score the skin reaction for erythema and oedema according to the scoring system given in Table 1 for each application site at each time interval. Record the appearance of each application site at (1±0.1) h, (24±2) h, (48±2) h and (72±2) h following removal of the patches.

Table 1 — Scoring system for skin reaction

Reaction	Irritation score
Erythema and Eschar Formation	
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
Oedema 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
Maximal possible score for irritation	8
Other adverse changes at the skin sites shall be recorded and reported.	

9.4 Evaluation of Results

Use only (24±2) h, (48±2) h and (72±2) h observations for calculation.

After the 72 h grading, all erythema grades plus oedema grades (24±2) h, (48±2) h and (72±2) h were totalled separately for each test sample and blank for each animal. The primary irritation score for an animal was calculated by dividing the sum of all the scores by 6 (two test/observation sites, three time points).

To obtain the primary irritation index for the test article, add all the primary irritation scores of the individual animals and divide by the number of animals.

When blank or negative control is used, calculate the primary irritation score for the controls and subtract that score from the score using the test material to obtain the primary irritation score.

The primary irritation index (PII) for the test article was evaluated according to Table 2.

Table 2 — Primary or cumulative irritation index categories in a rabbit

Mean score	Response category
0~0.4	Negligible
0.5~1.9	Slight
2~4.9	Moderate
5~8	Severe

10 Results

All animals were survived and no abnormal signs were observed during the study. According to what observed, the skin reaction of non-polar extract on testing side did not exceed that on the control side. Thus, the final test article score was calculated to be 0. See table 4.

11 Conclusion

The test result showed that the response of the test article extract was categorized as negligible under the test condition.

12 Record Storage

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

13 Confidentiality Agreement

Statements of confidentiality were as agreed upon prior to study initiation.

14 Deviation Statement

There were no deviations from the approved study protocol which were judged to have any impact on the validity of the data.

Annex 1 Test Data

Table 3 Positive control

Extract	Rabbit No.	Group	Reaction	Interval (hours): score=left site/right site 24±2h	48±2h	72±2h
SO	1	Positive Control	Erythema	2/3	3/3	4/3
			Oedema	3/3	4/4	4/4
		Negative Control	Erythema	0/0	0/0	0/0
			Oedema	0/0	0/0	0/0
SO	2	Positive Control	Erythema	3/3	3/3	4/3
			Oedema	3/3	3/4	4/4
		Negative Control	Erythema	0/0	0/0	0/0
			Oedema	0/0	0/0	0/0
SO	3	Positive Control	Erythema	3/3	4/3	4/4
			Oedema	3/2	3/3	3/3
		Negative Control	Erythema	0/0	0/0	0/0
			Oedema	0/0	0/0	0/0
The primary irritation score.					6.6	

Note：Positive control performed once every six months, see SDWH-M202003007-2(Completed Date: 2020-07-03).

Table 4 Test Results of Dermal Observations

Extract	Rabbit No.	Group	Reaction	Interval (hours): score=left site/right site 24±2h	48±2h	72±2h
SO	1	Test Article	Erythema	0/0	0/0	0/0
			Oedema	0/0	0/0	0/0
		Negative Control	Erythema	0/0	0/0	0/0
			Oedema	0/0	0/0	0/0
SO	2	Test Article	Erythema	0/0	0/0	0/0
			Oedema	0/0	0/0	0/0
		Negative Control	Erythema	0/0	0/0	0/0
			Oedema	0/0	0/0	0/0
SO	3	Test Article	Erythema	0/0	0/0	0/0
			Oedema	0/0	0/0	0/0
		Negative Control	Erythema	0/0	0/0	0/0
			Oedema	0/0	0/0	0/0
The primary irritation score.					0	

Annex 2 Photograph of Test Article

Annex 3 Information Provided by Sponsor

1 Production Process

Not supplied by sponsor.

2 Other Information

Not supplied by sponsor.

End of Report

180015144061

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检测
TESTING
CNAS L2954

Final Report

Report Number: SDWH-M202003265-2(E)

Skin Sensitization Test of Disposable Nitrile Examination Glove

According to ISO 10993-10:2010
Guinea Pig Maximization Test
0.9% Sodium Chloride Injection Extract

Sponsor: Shanxi Hongjin Plastic Technology Co., Ltd

Address: Coal Bed Gas Industrial Zone, Qu'e Town, Daning County, Linfen City, Shanxi Province

Sanitation & Environment Technology Institute, Soochow University
Address: 199 Ren-Ai Road, Suzhou Industrial Park, Suzhou, Jiangsu 215123, P. R. China
Website: www.sudatest.com E-mail: med@sudatest.com
Direct: +86 512 65880038 Free: 400 107 8828

Content

Supplementary Explanation

(1) Please apply for rechecking within 15 days of receiving the report if there are any objections.

(2) Any erasure or without special inspection and testing seal renders the report null and void.

(3) The report is only valid when signed by the persons who edited, checked and approved it.

(4) The results relate only to the articles tested.

(5) The report shall not be reproduced except in full without the written approval of the institute.

Quality Assurance Statement

The Quality Assurance Unit inspected/audited this study in compliance with the following GLP regulations:

Good Laboratory Practice (GLP) Regulation 21 CFR Part 58, U.S. Food and Drug Administration (FDA). The laboratory is exempt from the following provisions: 21 CFR Part 58.105 Test and Control Article Characterization, and Part 58.113 Mixtures of Articles with Carriers.

The Quality Assurance Unit conducted inspections on the following dates. The findings were reported to the Study Director and to the Testing Facility Management.

The final report was reviewed by the Quality Assurance Unit. The final report accurately describes the test methods in accordance with standard operating procedures, and the results are consistent with raw data of non-clinical studies conducted according to the study protocol.

Inspections	Date of Inspection	Date Reported to Study Director	Date Reported to Testing Facility Management.
Study Protocol	2020-06-30	2020-06-30	2020-08-18
Study Procedure	2020-07-24 2020-07-28	2020-07-24 2020-07-28	2020-08-18
Raw Data	2020-08-18	2020-08-18	2020-08-18
Final Report	2020-08-18	2020-08-18	2020-08-18

Quality Assurance Unit: Zou Jing 2020-08-18

Quality Assurance Date

GLP Compliance Statement

This study was fully in accordance with the technical requirements of the study protocol.

This study was conducted in compliance with Good Laboratory Practice (GLP) Regulation 21 CFR Part 58, U.S. Food and Drug Administration (FDA).

The laboratory is exempt from the following provisions: 21 CFR Part 58.105 Test and Control Article Characterization, and Part 58.113 Mixtures of Articles with Carriers.

Verification Dates

Test Article Receipt	2020-06-23
Protocol Effective Date	2020-06-30
Technical Initiation Date	2020-06-30
Technical Completion Date	2020-07-31
Final Report Completion Date	2020-08-18

Edited by: Wang Deheng 2020-08-17
Date

Reviewed by: Zhang Yan 2020-08-18
Study Director Date

Approved by: Fangjingxi 2020-08-18
Authorized Signatory Date

Sanitation & Environment Technology Institute, Soochow University

Summary

1 Test Article

Test Article Name	Disposable Nitrile Examination Glove
Manufacturer	Shanxi Hongjin Plastic Technology Co., Ltd
Address	Coal Bed Gas Industrial Zone, Qu'e Town, Daning County, Linfen City, Shanxi Province
Model	Not supplied by sponsor (N/S)
Lot/Batch	N/S

2 Main Reference

ISO 10993-10:2010 Biological evaluation of medical devices — Part 10: Tests for irritation and skin sensitization

3 Test Method

Potential skin sensitization of test article was evaluated using guinea pig maximization test in accordance with ISO 10993-10:2010 Biological evaluation of medical devices — Part 10: Tests for irritation and skin sensitization.
Study protocol number: SDWH-PROTOCOL-GLP-M202003265-2.

4 Conclusion

Under the conditions of this study, the test article extract showed no significant evidence of causing skin sensitization in the guinea pig. The positive rate of sensitization was 0%. No evidence of skin sensitization in guinea pigs was found.

Test Report

1 Purpose

The test was designed to evaluate the potential of a test article to cause skin sensitization. The test is used as a procedure for screening of contact allergens in guinea pigs and extrapolating the results to humans, but it does not establish the actual risk of sensitization.

2 Reference

ISO 10993-10:2010 Biological evaluation of medical devices — Part 10: Tests for irritation and skin sensitization
ISO 10993-12:2012 Biological evaluation of medical devices — Part 12: Sample preparation and reference materials
ISO 10993-2:2006 Biological evaluation of medical devices — Part 2: Animal welfare requirements

3 Compliance

Good Laboratory Practice Regulations, 21 CFR, Part 58.
ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories (CNAS—CL01 Accreditation criteria for the competence of testing and calibration laboratories) China National Accreditation Service for Conformity Assessment LABORATORY ACCREDITATION CERTIFICATE Registration No. CNAS L2954.
RB/T 214—2017 Competence assessment for inspection body and laboratory mandatory approval—General requirements for inspection body and laboratory Certification and Accreditation Administration of the People's Republic of China INSPECTION BODY AND LABORATORY MANDATORY APPROVAL Certificate No. CMA 180015144061.

4 Identification of Test and Control Articles

4.1 Test Article

Test Article Name	Disposable Nitrile Examination Glove
Manufacturer	Shanxi Hongjin Plastic Technology Co., Ltd
Address	Coal Bed Gas Industrial Zone, Qu'e Town, Daning County, Linfen City, Shanxi Province
Test Article Initial State	Not Sterilized
CAS Code	N/S
Model	N/S
Size	M
Lot/Batch	N/S
Test Article Material	nitrile
Packaging Material	N/S
Physical State	pieces
Color	blue
Density	N/S
Stability	N/S
Solubility	N/S
Storage Condition	Room Temperature
Intended Clinical Use	to prevent cross contamination

The information about the test article was supplied by the sponsor wherever applicable.

The Sponsor is responsible for all test article characterization data as specified in the GLP regulations.

4.2 Control Article

4.2.1 Negative Control

Article Name: 0.9% Sodium Chloride Injection (SC)
Manufacturer: Guangxi Yuyuan Pharmaceutical Co., Ltd.
Size: 500mL
Lot/ Batch#: H20010204
Physical State: Liquid
Color: Colorless
Storage Condition: Room Temperature

4.2.2 Positive Control

Article Name: 2, 4-Dinitrochlorobenzene (DNCB)
Manufacturer: Chengdu Aikeda Chemical Reagent Co., Ltd.
Size: 100g
Lot/ Batch#: 201904101
Induction Concentration: 0.5%
Challenge Concentration: 0.1%
Solvent: 0.9% Sodium Chloride Injection
Date prepared: Intradermal Induction Phase I :2020-06-08; Topical Induction Phase II: 2020-06-15;
Challenge Phase: 2020-06-29
Physical State: Liquid
Color: Light Yellow
Storage Condition: Room Temperature

5 Equipment and Reagents

5.1 Equipment

Equipment Name	Equipment Number	Calibration Expire
Horizontal Large Capacity Constant Temperature Vibrator	SDWH2671	2021-01-13
Vertical pressure steam sterilizer	SDWH2097	2021-03-25
Steel straight scale	SDWH463	2020-07-29
Electronic scale	SDWH442	2021-04-25

5.2 Reagents

Reagent Name	Manufacturer	LOT
Freund's adjuvant, complete liquid	SIGMA	SLCC3348
Sodium dodecyl sulfate (SDS)	Sinopharm Chemical Reagent Co., Ltd	20181210

6 Identification of Test System

Species: Hartley guinea pig (Cavia Porcellus)
Number: 15 (10 test +5 negative control)
Sex: Male
Initial body weight: 300 ~ 500 g
Health status: healthy, not previously used in other experimental procedures
Housing: animals were housed in groups in cages identified by a card indicating the lab number,

test code and first treatment date, etc.
Animal identification: Stain with dyeing liquid
Cages: plastic cage
Acclimation period: 7 days under the same conditions as for the actual test

7 Animal Care and Maintenance

Animal source: Shanghai Jia Gan Biotechnology Co., Ltd. <Permit Code: SCXK (HU) 2015-0005>
Bedding: corncob, Suzhou Shuangshi Laboratory Animal Feed Science Co., Ltd.
Feed: guinea pig diet, Suzhou Experimental Animal Sci-Tech Co., Ltd.
Water: drinking water met the Standards for Drinking Water Quality GB 5749–2006
Animal room temperature: 18 ~ 26°C
Animal room relative humidity: 30% ~ 70%
Lights: 12 h light/dark cycle, full-spectrum lighting
Personnel: associates involved were appropriately qualified and trained
Selection: only healthy, previously unused animals were selected
There were no known contaminants present in the feed, water, or bedding expected to interfere with the test data.

8 Justification of Test System and Route of Administration

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, 2,4-dinitrochlorobenzene (DNCB) has been substantiated at SDWH (listed in **Table 1** and **Table 2**).
The test article was extracted and administered in vivo through a medium compatible with the test system. Dermal application corresponds to the likely route of human exposure.

9 Experimental Design

9.1 Preparation of Extracts

9.1.1 Pretreatment

Autoclaving at 121°C for 30 min.

9.1.2 Extraction

Under aseptic conditions, samples were taken according to the sampling method (Random sampling). The extraction was performed with agitation in closed inert containers according to the extraction ratio listed in the following table (sample: extraction vehicle). The extraction vehicle was 0.9% Sodium Chloride Injection (SC).

Test Period	Actual Sampling	Extract Procedure			Final Extract
		Extract Ratio	SC	Condition	
Intradermal Induction Phase I	Surface area 120 cm^2	6 cm^2 : 1 mL	20.0 mL	50°C, 72 h	Clear
Topical Induction Phase II	Surface area 120 cm^2	6 cm^2 : 1 mL	20.0 mL	50°C, 72 h	Clear
Challenge Phase	Surface area 120 cm^2	6 cm^2 : 1 mL	20.0 mL	50°C, 72 h	Clear

The state of the extract did not change after extraction. The extract was stored at room temperature, and tested within 24 h, without the process of adjusting its pH value, filtering, centrifuging, diluting, etc.
The vehicle (without the test article) was similarly prepared to serve as the control.

9.2 Experimental Procedure

9.2.1 Animal Preparation and Grouping

On the first day of treatment, 15 guinea pigs were weighed and identified. The fur from the dorsoscapular area of the animals was removed with an electric clipper. Grouping as follow:

Group Name	Group Size	Gender
Test	10 animals	Male
Negative Control	5 animals	Male

9.2.2 Intradermal Induction Phase I

A pair of 0.1 mL 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.

Site A: A 50:50 (*V*/*V*) 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 (*V*/*V*) stable emulsion of Freund's complete adjuvant and the solvent (50%); the control animals were injected with an emulsion of the blank liquid with adjuvant.

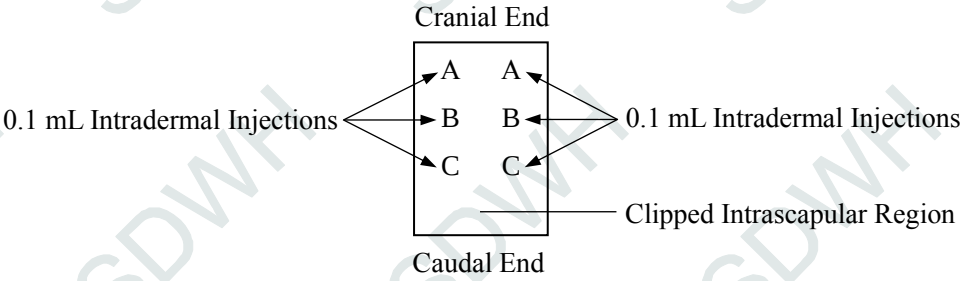

Figure 1 Locations of intradermal injection sites

9.2.3 Topical Induction Phase II

The maximum concentration that can be achieved in Intradermal induction phase I did not produce irritation. Animals are pretreated with 10% sodium dodecyl sulfate (Solvent: Distilled water, Date prepared: 2020-03-25) (24 ± 2) h before the topical induction application.

At 7 ± 1 d after completion of the intradermal induction phase, administer 0.5 mL test article extract by topical application to the intrascapular region of each animal, using a patch of area approximately 8 cm^2 (absorbent gauze), so as to cover the intradermal injection sites. Secure the patches with an occlusive dressing. Remove the dressings and patches after (48 ± 2) h.

Treat the control animals similarly, using the blank liquid alone.

9.2.4 Challenge Phase

At 14 ± 1 d after completion of the topical induction phase, challenge all test and control animals with the test sample. Absorbent gauzes (2.5 cm × 2.5 cm) were soaked respectively with 0.5 mL test article and 0.5 mL control article. Apply the test article extract and control article topically to two sites that were not treated during the induction stage. Secure with an occlusive dressing. Remove the dressings and patches after (24 ± 2) h.

9.3 Observation of Animals

Observe the appearance of the challenge skin sites of the test and control animals (24 ± 2) h and (48 ± 2) h after removal of the dressings. Full-spectrum lighting was used to visualize the skin reactions. Describe and grade the skin reactions for erythema and oedema according to the Magnusson and Kligman grading given in the following table for each challenge site and at each

time interval.

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/or swelling	3

9.4 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 animals.
If grades of 1 or greater are noted in control animals, then the reactions of test animals which exceed the most severe reaction in control animals are presumed to be due to sensitization.
If the response is equivocal, rechallenge 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 test and control animals.

10 Results

The results of skin reaction after challenge were listed in **Table 3**. No skin sensitization reaction was found in the skin of guinea pigs using extracts of the test article, and the positive rate of sensitization was 0%.
The positive rate of sensitization in the positive control group was 100%, listed in **Table 1**.
Clinical observations and weight changes of guinea pigs were listed in **Table 4.**

11 Conclusion

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

12 Record Storage

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

13 Confidentiality Agreement

Statements of confidentiality were as agreed upon prior to study initiation.

14 Deviation Statement

There were no deviations from the approved study protocol which were judged to have any impact on the validity of the data.

Annex 1 Test Data

Table 1 Guinea pig sensitization dermal reactions of positive control

Group	Animal Number	(24 ± 2) h Before Phase II Patch Application		(24 ± 2) h Following Challenge Phase		(48 ± 2) h Following Challenge Phase		Positive Rate after Challenge Phase
		Left	Right	Test Sites	Control Sites	Test Sites	Control Sites	
Positive Control	1	2	3	1	0	2	0	100%
	2	2	2	2	0	2	0	
	3	2	2	2	0	1	0	
	4	2	2	2	0	1	0	
	5	2	3	1	0	1	0	
Negative Control	6	0	0	0	0	0	0	-
	7	0	0	0	0	0	0	
	8	0	0	0	0	0	0	
	9	0	0	0	0	0	0	
	10	0	0	0	0	0	0	

Note: the data of positive control come from SDWH- M202002662-1 (Completed Date: 2020-07-03)

Table 2 Weigh change and clinical observation of positive control

Group	Animal Number	Weight (g)		Clinical Observation Except Dermal Reactions
		Before Injection	After Experiment	
Positive Control	1	307	371	Normal
	2	348	428	Normal
	3	329	407	Normal
	4	355	440	Normal
	5	311	379	Normal
Negative Control	6	324	396	Normal
	7	347	433	Normal
	8	313	383	Normal
	9	341	417	Normal
	10	305	372	Normal

Note: the data of positive control come from SDWH- M202002662-1 (Completed Date: 2020-07-03)

Table 3 Guinea pig sensitization dermal reactions

Group	Animal Number	(24 ± 2) h Before Phase II Patch Application		(24 ± 2) h Following Challenge Phase		(48 ± 2) h Following Challenge Phase		Positive Rate after Challenge Phase
		Left	Right	Test Sites	Control Sites	Test Sites	Control Sites	
Test	1	0	0	0	0	0	0	0%
	2	0	0	0	0	0	0	
	3	0	0	0	0	0	0	
	4	0	0	0	0	0	0	
	5	0	0	0	0	0	0	
	6	0	0	0	0	0	0	
	7	0	0	0	0	0	0	
	8	0	0	0	0	0	0	
	9	0	0	0	0	0	0	
	10	0	0	0	0	0	0	
Negative Control	11	0	0	0	0	0	0	-
	12	0	0	0	0	0	0	
	13	0	0	0	0	0	0	
	14	0	0	0	0	0	0	
	15	0	0	0	0	0	0	

Table 4 Weigh change and clinical observation

Group	Animal Number	Weight (g)		Clinical Observation Except Dermal Reactions
		Before Injection	After Experiment	
Test	1	339	414	Normal
	2	322	392	Normal
	3	353	441	Normal
	4	352	440	Normal
	5	322	398	Normal
	6	308	370	Normal
	7	356	440	Normal
	8	349	439	Normal
	9	333	413	Normal
	10	319	387	Normal
Negative Control	11	337	416	Normal
	12	343	430	Normal
	13	353	439	Normal
	14	316	383	Normal
	15	312	376	Normal

Annex 2 Photograph of Test Article

Annex 3 Information Provided by Sponsor

1 Production Process

Not supplied by sponsor.

2 Other Information

Not supplied by sponsor.

End of Report

180015144061

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Final Report

Report Number: SDWH-M202003265-3(E)

Skin Sensitization Test of Disposable Nitrile Examination Glove

According to ISO 10993-10:2010
Guinea Pig Maximization Test
Sesame Oil Extract

Sponsor: Shanxi Hongjin Plastic Technology Co., Ltd

Address: Coal Bed Gas Industrial Zone, Qu'e Town, Daning County, Linfen City, Shanxi Province

Sanitation & Environment Technology Institute, Soochow University
Address: 199 Ren-Ai Road, Suzhou Industrial Park, Suzhou, Jiangsu 215123, P. R. China
Website: www.sudatest.com E-mail: med@sudatest.com
Direct: +86 512 65880038 Free: 400 107 8828

Content

Supplementary Explanation

(1) Please apply for rechecking within 15 days of receiving the report if there are any objections.

(2) Any erasure or without special inspection and testing seal renders the report null and void.

(3) The report is only valid when signed by the persons who edited, checked and approved it.

(4) The results relate only to the articles tested.

(5) The report shall not be reproduced except in full without the written approval of the institute.

Quality Assurance Statement

The Quality Assurance Unit inspected/audited this study in compliance with the following GLP regulations:

Good Laboratory Practice (GLP) Regulation 21 CFR Part 58, U.S. Food and Drug Administration (FDA). The laboratory is exempt from the following provisions: 21 CFR Part 58.105 Test and Control Article Characterization, and Part 58.113 Mixtures of Articles with Carriers.

The Quality Assurance Unit conducted inspections on the following dates. The findings were reported to the Study Director and to the Testing Facility Management.

The final report was reviewed by the Quality Assurance Unit. The final report accurately describes the test methods in accordance with standard operating procedures, and the results are consistent with raw data of non-clinical studies conducted according to the study protocol.

Inspections	Date of Inspection	Date Reported to Study Director	Date Reported to Testing Facility Management.
Study Protocol	2020-06-30	2020-06-30	2020-08-18
Study Procedure	2020-07-24 2020-07-28	2020-07-24 2020-07-28	2020-08-18
Raw Data	2020-08-18	2020-08-18	2020-08-18
Final Report	2020-08-18	2020-08-18	2020-08-18

Quality Assurance Unit: Zou Jing 2020-08-18

Quality Assurance Date

GLP Compliance Statement

This study was fully in accordance with the technical requirements of the study protocol.

This study was conducted in compliance with Good Laboratory Practice (GLP) Regulation 21 CFR Part 58, U.S. Food and Drug Administration (FDA).

The laboratory is exempt from the following provisions: 21 CFR Part 58.105 Test and Control Article Characterization, and Part 58.113 Mixtures of Articles with Carriers.

Verification Dates

Test Article Receipt	2020-06-23
Protocol Effective Date	2020-06-30
Technical Initiation Date	2020-06-30
Technical Completion Date	2020-07-31
Final Report Completion Date	2020-08-18

Edited by: Wang Deheng 2020-08-17
Date

Reviewed by: Zhang Yan 2020-08-18
Study Director Date

Approved by: Fangjingxi 2020-08-18
Authorized Signatory Date

Sanitation & Environment Technology Institute, Soochow University

Summary

1 Test Article

Test Article Name	Disposable Nitrile Examination Glove
Manufacturer	Shanxi Hongjin Plastic Technology Co., Ltd
Address	Coal Bed Gas Industrial Zone, Qu'e Town, Daning County, Linfen City, Shanxi Province
Model	Not supplied by sponsor (N/S)
Lot/Batch	N/S

2 Main Reference

ISO 10993-10:2010 Biological evaluation of medical devices — Part 10: Tests for irritation and skin sensitization

3 Test Method

Potential skin sensitization of test article was evaluated using guinea pig maximization test in accordance with ISO 10993-10:2010 Biological evaluation of medical devices — Part 10: Tests for irritation and skin sensitization.
Study protocol number: SDWH-PROTOCOL-GLP-M202003265-3.

4 Conclusion

Under the conditions of this study, the test article extract showed no significant evidence of causing skin sensitization in the guinea pig. The positive rate of sensitization was 0%. No evidence of skin sensitization in guinea pigs was found.

Test Report

1 Purpose

The test was designed to evaluate the potential of a test article to cause skin sensitization. The test is used as a procedure for screening of contact allergens in guinea pigs and extrapolating the results to humans, but it does not establish the actual risk of sensitization.

2 Reference

ISO 10993-10:2010 Biological evaluation of medical devices — Part 10: Tests for irritation and skin sensitization
ISO 10993-12:2012 Biological evaluation of medical devices — Part 12: Sample preparation and reference materials
ISO 10993-2:2006 Biological evaluation of medical devices — Part 2: Animal welfare requirements

3 Compliance

Good Laboratory Practice Regulations, 21 CFR, Part 58.
ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories (CNAS—CL01 Accreditation criteria for the competence of testing and calibration laboratories) China National Accreditation Service for Conformity Assessment LABORATORY ACCREDITATION CERTIFICATE Registration No. CNAS L2954.
RB/T 214—2017 Competence assessment for inspection body and laboratory mandatory approval—General requirements for inspection body and laboratory Certification and Accreditation Administration of the People's Republic of China INSPECTION BODY AND LABORATORY MANDATORY APPROVAL Certificate No. CMA 180015144061.

4 Identification of Test and Control Articles

4.1 Test Article

Test Article Name	Disposable Nitrile Examination Glove
Manufacturer	Shanxi Hongjin Plastic Technology Co., Ltd
Address	Coal Bed Gas Industrial Zone, Qu'e Town, Daning County, Linfen City, Shanxi Province
Test Article Initial State	Not Sterilized
CAS Code	N/S
Model	N/S
Size	M
Lot/Batch	N/S
Test Article Material	nitrile
Packaging Material	N/S
Physical State	pieces
Color	blue
Density	N/S
Stability	N/S
Solubility	N/S
Storage Condition	Room Temperature
Intended Clinical Use	to prevent cross contamination

The information about the test article was supplied by the sponsor wherever applicable.

The Sponsor is responsible for all test article characterization data as specified in the GLP regulations.

4.2 Control Article

4.2.1 Negative Control

Article Name: Sesame oil (SO).
Manufacturer: Ji'an Qingyuan District luyuanxiangliao. Co. Ltd
Size: 5kg
Lot/ Batch#: 20200312
Physical State: Oily liquid
Color: Pale yellow
Storage Condition: Room Temperature

4.2.2 Positive Control

Article Name: 2, 4-Dinitrochlorobenzene (DNCB)
Manufacturer: Chengdu Aikeda Chemical Reagent Co., Ltd.
Size: 100g
Lot/ Batch#: 201904101
Induction Concentration: 0.5%
Challenge Concentration: 0.1%
Solvent: Sesame oil
Date prepared: Intradermal Induction Phase I :2020-06-08; Topical Induction Phase II: 2020-06-15;
Challenge Phase: 2020-06-29
Physical State: Liquid
Color: Light Yellow
Storage Condition: Room Temperature

5 Equipment and Reagents

5.1 Equipment

Equipment Name	Equipment Number	Calibration Expire
Horizontal Large Capacity Constant Temperature Vibrator	SDWH2671	2021-01-13
Vertical pressure steam sterilizer	SDWH2097	2021-03-25
Steel straight scale	SDWH463	2020-07-29
Electronic scale	SDWH442	2021-04-25

5.2 Reagents

Reagent Name	Manufacturer	LOT
Freund's adjuvant, complete liquid	SIGMA	SLCC3348
Sodium dodecyl sulfate (SDS)	Sinopharm Chemical Reagent Co., Ltd	20181210

6 Identification of Test System

Species: Hartley guinea pig (Cavia Porcellus)
Number: 15 (10 test +5 negative control)
Sex: Male
Initial body weight: 300 ~ 500 g
Health status: healthy, not previously used in other experimental procedures
Housing: animals were housed in groups in cages identified by a card indicating the lab number,

test code and first treatment date, etc.
Animal identification: Stain with dyeing liquid
Cages: plastic cage
Acclimation period: 7 days under the same conditions as for the actual test

7 Animal Care and Maintenance

Animal source: Shanghai Jia Gan Biotechnology Co., Ltd. <Permit Code: SCXK (HU) 2015-0005>
Bedding: corncob, Suzhou Shuangshi Laboratory Animal Feed Science Co., Ltd.
Feed: guinea pig diet, Suzhou Experimental Animal Sci-Tech Co., Ltd.
Water: drinking water met the Standards for Drinking Water Quality GB 5749–2006
Animal room temperature: 18 ~ 26°C
Animal room relative humidity: 30% ~ 70%
Lights: 12 h light/dark cycle, full-spectrum lighting
Personnel: associates involved were appropriately qualified and trained
Selection: only healthy, previously unused animals were selected
There were no known contaminants present in the feed, water, or bedding expected to interfere with the test data.

8 Justification of Test System and Route of Administration

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, 2,4-dinitrochlorobenzene (DNCB) has been substantiated at SDWH (listed in **Table 1** and **Table 2**).
The test article was extracted and administered in vivo through a medium compatible with the test system. Dermal application corresponds to the likely route of human exposure.

9 Experimental Design

9.1 Preparation of Extracts

9.1.1 Pretreatment

Autoclaving at 121°C for 30 min.

9.1.2 Extraction

Under aseptic conditions, samples were taken according to the sampling method (Random sampling). The extraction was performed with agitation in closed inert containers according to the extraction ratio listed in the following table (sample: extraction vehicle). The extraction vehicle was SO.

Test Period	Actual Sampling	Extract Procedure			Final Extract
		Extract Ratio	SO	Condition	
Intradermal Induction Phase I	Surface area 120 cm^2	6 cm^2 : 1 mL	20.0 mL	50°C, 72 h	Clear
Topical Induction Phase II	Surface area 120 cm^2	6 cm^2 : 1 mL	20.0 mL	50°C, 72 h	Clear
Challenge Phase	Surface area 120 cm^2	6 cm^2 : 1 mL	20.0 mL	50°C, 72 h	Clear

The state of the extract did not change after extraction. The extract was stored at room temperature, and tested within 24 h, without the process of adjusting its pH value, filtering, centrifuging, diluting, etc.
The vehicle (without the test article) was similarly prepared to serve as the control.

9.2 Experimental Procedure

9.2.1 Animal Preparation and Grouping

On the first day of treatment, 15 guinea pigs were weighed and identified. The fur from the dorsoscapular area of the animals was removed with an electric clipper. Grouping as follow:

Group Name	Group Size	Gender
Test	10 animals	Male
Negative Control	5 animals	Male

9.2.2 Intradermal Induction Phase I

A pair of 0.1 mL 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.

Site A: A 50:50 (*V*/*V*) 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 (*V*/*V*) stable emulsion of Freund's complete adjuvant and the solvent (50%); the control animals were injected with an emulsion of the blank liquid with adjuvant.

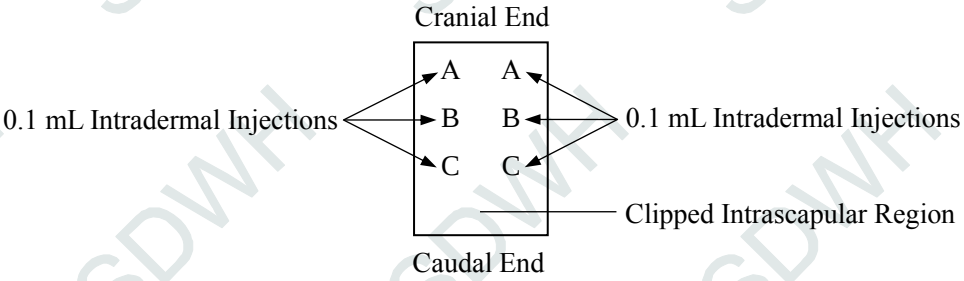

Figure 1 Locations of intradermal injection sites

9.2.3 Topical Induction Phase II

The maximum concentration that can be achieved in Intradermal induction phase I did not produce irritation. Animals are pretreated with 10% sodium dodecyl sulfate (Solvent: Distilled water, Date prepared: 2020-03-25) (24 ± 2) h before the topical induction application.

At 7 ± 1 d after completion of the intradermal induction phase, administer 0.5 mL test article extract by topical application to the intrascapular region of each animal, using a patch of area approximately 8 cm^2 (absorbent gauze), so as to cover the intradermal injection sites. Secure the patches with an occlusive dressing. Remove the dressings and patches after (48 ± 2) h.

Treat the control animals similarly, using the blank liquid alone.

9.2.4 Challenge Phase

At 14 ± 1 d after completion of the topical induction phase, challenge all test and control animals with the test sample. Absorbent gauzes (2.5 cm × 2.5 cm) were soaked respectively with 0.5 mL test article and 0.5 mL control article. Apply the test article extract and control article topically to two sites that were not treated during the induction stage. Secure with an occlusive dressing. Remove the dressings and patches after (24 ± 2) h.

9.3 Observation of Animals

Observe the appearance of the challenge skin sites of the test and control animals (24 ± 2) h and (48 ± 2) h after removal of the dressings. Full-spectrum lighting was used to visualize the skin reactions. Describe and grade the skin reactions for erythema and oedema according to the Magnusson and Kligman grading given in the following table for each challenge site and at each

time interval.

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/or swelling	3

9.4 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 animals.
If grades of 1 or greater are noted in control animals, then the reactions of test animals which exceed the most severe reaction in control animals are presumed to be due to sensitization.
If the response is equivocal, rechallenge 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 test and control animals.

10 Results

The results of skin reaction after challenge were listed in **Table 3**. No skin sensitization reaction was found in the skin of guinea pigs using extracts of the test article, and the positive rate of sensitization was 0%.
The positive rate of sensitization in the positive control group was 100%, listed in **Table 1**.
Clinical observations and weight changes of guinea pigs were listed in **Table 4.**

11 Conclusion

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

12 Record Storage

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

13 Confidentiality Agreement

Statements of confidentiality were as agreed upon prior to study initiation.

14 Deviation Statement

There were no deviations from the approved study protocol which were judged to have any impact on the validity of the data.

Annex 1 Test Data

Table 1 Guinea pig sensitization dermal reactions of positive control

Group	Animal Number	(24 ± 2) h Before Phase II Patch Application		(24 ± 2) h Following Challenge Phase		(48 ± 2) h Following Challenge Phase		Positive Rate after Challenge Phase
		Left	Right	Test Sites	Control Sites	Test Sites	Control Sites	
Positive Control	1	1	2	1	0	2	0	100%
	2	2	2	2	0	2	0	
	3	2	1	1	0	2	0	
	4	2	2	2	0	1	0	
	5	2	3	2	0	1	0	
Negative Control	6	0	0	0	0	0	0	-
	7	0	0	0	0	0	0	
	8	0	0	0	0	0	0	
	9	0	0	0	0	0	0	
	10	0	0	0	0	0	0	

Note: the data of positive control come from SDWH- M202002662-2 (Completed Date: 2020-07-03)

Table 2 Weigh change and clinical observation of positive control

Group	Animal Number	Weight (g)		Clinical Observation Except Dermal Reactions
		Before Injection	After Experiment	
Positive Control	1	341	417	Normal
	2	345	431	Normal
	3	355	448	Normal
	4	311	375	Normal
	5	354	447	Normal
Negative Control	6	335	414	Normal
	7	319	389	Normal
	8	336	416	Normal
	9	356	441	Normal
	10	343	429	Normal

Note: the data of positive control come from SDWH- M202002662-2 (Completed Date: 2020-07-03)

Table 3 Guinea pig sensitization dermal reactions

Group	Animal Number	(24 ± 2) h Before Phase II Patch Application		(24 ± 2) h Following Challenge Phase		(48 ± 2) h Following Challenge Phase		Positive Rate after Challenge Phase
		Left	Right	Test Sites	Control Sites	Test Sites	Control Sites	
Test	1	0	0	0	0	0	0	0%
	2	0	0	0	0	0	0	
	3	0	0	0	0	0	0	
	4	0	0	0	0	0	0	
	5	0	0	0	0	0	0	
	6	0	0	0	0	0	0	
	7	0	0	0	0	0	0	
	8	0	0	0	0	0	0	
	9	0	0	0	0	0	0	
	10	0	0	0	0	0	0	
Negative Control	11	0	0	0	0	0	0	-
	12	0	0	0	0	0	0	
	13	0	0	0	0	0	0	
	14	0	0	0	0	0	0	
	15	0	0	0	0	0	0	

Table 4 Weigh change and clinical observation

Group	Animal Number	Weight (g)		Clinical Observation Except Dermal Reactions
		Before Injection	After Experiment	
Test	1	318	387	Normal
	2	346	434	Normal
	3	320	388	Normal
	4	328	404	Normal
	5	314	386	Normal
	6	333	411	Normal
	7	319	386	Normal
	8	343	424	Normal
	9	330	400	Normal
	10	333	406	Normal
Negative Control	11	315	379	Normal
	12	341	426	Normal
	13	350	431	Normal
	14	337	415	Normal
	15	309	371	Normal

Annex 2 Photograph of Test Article

Annex 3 Information Provided by Sponsor

1 Production Process

Not supplied by sponsor.

2 Other Information

Not supplied by sponsor.

End of Report