191020340175

中国认可
国际互认
检测
TESTING
CNAS L13034

Skin Irritation Test

Extraction Method

Final Report

Article Name: Disposable surgical face mask

Report Number: CSTBB20030404

Method Standard: ISO 10993-10: 2010

Sponsor

Hunan EEXI Technology&Service Co.,Ltd.

No.6, North of Pingtou road, Liuyang Hi-tech industrial development zone, Hunan, China

Test Facility

CCIC Huatongwei international inspection (Suzhou) Co., Ltd

Room 101, Building G, Ruoshui Road 388, Suzhou, Jiangsu, China

CONTENTS

Notices

1. Please apply for rechecking within 15 days of receiving the report if there is any objection.
2. Any erasure or without special 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 report is only responsible for the test results of the tested samples.
5. The report shall not be reproduced except in full without the written approval of the company.

Abstract

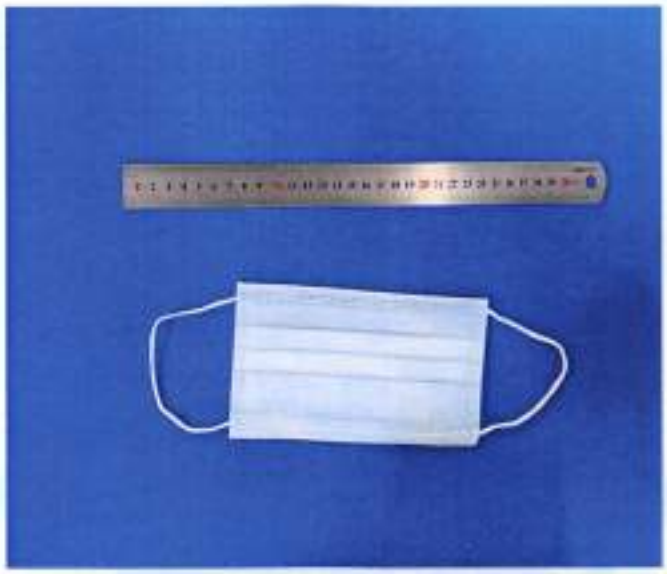
In this study, we took New Zealand white Rabbits to observe the skin irritation of the test article according to ISO10993-10:2010.

The test article were extracted in Constant Temperature Vibrator at 50 ℃, 60 rpm for 72 h by 0.9 % Sodium Chloride Injection and Sesame Oil. Apply 0.5 ml extracts 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, and then wrap the application sites with a bandage for a minimum of 4 h. At the end of the contact time, remove the dressing. The describe and score the skin reaction for erythema and oedema 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.

The results showed that the rabbits in the negative control group (0.9 % Sodium Chloride Injection, Sesame Oil) retained a normal appearance throughout the test and showed no skin irritants. A severe skin reactions for erythema and oedema were shown in the positive control group (SDS). While in test article group, the response of skin on testing side did not exceed that on the control side. The skin reactions for erythema and oedema were not observed in test article group. The data of each group met the acceptance criteria, and the results of this test were considered valid.

Based on the above results, it can be concluded that under the experimental conditions, the test article Disposable surgical face mask has no potential skin irritation on rabbit in the extraction method.

Study Verification and Signature



Protocol Number	SST2003015902BB
Protocol Effective Date	2020-03-23
Technical Initiation Date	2020-03-27
Technical Completion Date	2020-04-03
Final Report Completion Date	2020-05-11

Personnel _____ 2020-05-11
Date Completed

Approved _____ 2020-05-11
Study Director — Date Completed

Supervisory _____
Test Facility Manager — Date Completed

Huatongwei international inspection (Suzhou) Co., Ltd.

1.0 Purpose

To evaluate the potential skin irritation caused by test article contact with the skin surface of rabbits and extrapolating the results to humans, but it does not establish the actual risk of irritation.

2.0 Reference

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

Biological evaluation of medical devices-Part 12: Sample preparation and reference materials (ISO 10993-12:2012)

Biological evaluation of medical devices-Part 2: Animal welfare requirements (ISO 10993-2:2006)

3.0 Test and control articles

Groups	Test article	Negative Control Article(Polar)	Negative Control Article(Non-Polar)	Positive Control
Name	Disposable surgical face mask	Sodium Chloride Injection (SC)	Sesame Oil (SO)	10 % sodium dodecyl sulfate (SDS)
Manufacture	Hunan EEXI Technology&Service Co.,Ltd.	Shijiazhuang No.4 Pharmaceutical	Jiangxi xinsen natural vegetable oil co., Ltd.	SIGMA
Size	17.5cm*9.5cm	500 ml	25 kg	25 g
Model	YX011	/	/	/
Lot Batch#	Not provided	1912121907	181120	SLBL2304V
Test Article Material	Not provided	/	/	/
Physical State	Solid	Liquid	Liquid	Solid
Color	Not provided	Colorless	Light yellow	Colorless
Package material	Not provided	/	/	/
Sterilization state	No	/	/	/
Concentration	/	0.9 %	/	10 %
Total Surface/Weight	Not provided	/	/	/
Storage Condition	Room Tep.	Room Tep.	Room Tep.	Room Tep.
The information about the test article was supplied by the sponsor wherever applicable.				

4.0 Identification of test system

4.1 Test animal

Species: New Zealand white Rabbit

Number: 6

Sex: 3 ♀, 3 ♂

Weight: 2.05~2.21 kg

Health status: Healthy, not previously used in other experimental procedures

Animal identification: Ear tattoo

Cages: Stainless steel cage

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

4.2 Justification of test system

The rabbit is specified as an appropriate animal model for evaluating potential skin irritants by the c urrent testing standards. Positive control 10% sodium dodecyl sulfate has been substantiated at HTW with this method.

5.0 Animal Managment

Animal purchase: Wuxi hengtai experimental animal breeding co. LTD SCXK (SU) 2015-0004

Bedding: /

Feed: Experimental rabbits were fed a maintenance diet, Wuxi hengtai experimental animal breeding co. LTD

Water: Drinking water met the Standards for Drinking Water Quality GB 5749-2006

Animal room temperature: 18-26 ℃

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, or bedding expected to interfere with the test data

6.0 Equipment and reagents

6.1 Instruments

Constant Temperature Vibrator (SHB007, calibration data: 2020/3/16), Autoclave (SHB026, calibration data: 2020/3/16), Electronic scale (SHB017, calibration data: 2020/3/16)

7.0 Experiment design

7.1 Sample preparation

The extracts of test article will be prepared according to the following steps:

Aseptic Sampling			Extraction in sterile vessels				
Sampling Manner	Actually sampling	Ratio	Reagent		Temperature	Time	pH
Whole	570.0 cm^2	6 cm^2: 1 ml	SC	95.0 ml	50 ℃	72 h	5.5
	570.0 cm^2		SO	95.0 ml			5.5

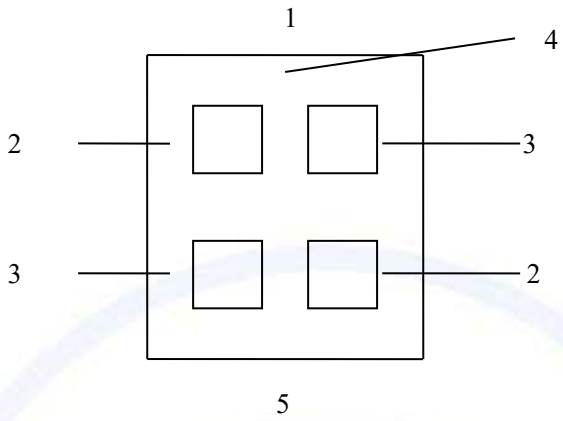
The state of the leaching solution did not change visually after the leaching was advanced. The extractions were clear, and the pH value has not been adjusted, filtered, centrifuged, diluted and other processes, before dosing stored at room temperature no more than 24 h. The control solution was prepared under the same conditions

7.2 Test method

Use the rabbits with healthy intact skin. Fur was generally clipped within 24 h period before 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 10×15 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 4h. At the end of the contact time, remove the dressing.



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

Figure1 Location of skin application sites

8.0 The results observed

The 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 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
Maximal 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

9.0 Evaluation criteria

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 article 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 was 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.

10.0 Results of the test

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 2.

11.0 Conclusion

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

12.0 Record

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

13.0 Confidentiality Agreement

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

Table 2　Skin irritation response observation

Reagent	Rabbit No	Pretest weigh(g)	Finished weigh(g)	Group	Reaction	Interval (hours): score=left/right			
						1h	24h	48h	72h
SC	1	2.16	2.25	Test Article	Erythema	0/0	0/0	0/0	0/0
					Oedema	0/0	0/0	0/0	0/0
				Negative Control	Erythema	0/0	0/0	0/0	0/0
					Oedema	0/0	0/0	0/0	0/0
	2	2.05	2.15	Test Article	Erythema	0/0	0/0	0/0	0/0
					Oedema	0/0	0/0	0/0	0/0
				Negative Control	Erythema	0/0	0/0	0/0	0/0
					Oedema	0/0	0/0	0/0	0/0
	3	2.17	2.26	Test Article	Erythema	0/0	0/0	0/0	0/0
					Oedema	0/0	0/0	0/0	0/0
				Negative Control	Erythema	0/0	0/0	0/0	0/0
					Oedema	0/0	0/0	0/0	0/0
Primary irritation index						0			
SO	4	2.18	2.26	Test Article	Erythema	0/0	0/0	0/0	0/0
					Oedema	0/0	0/0	0/0	0/0
				Negative Control	Erythema	0/0	0/0	0/0	0/0
					Oedema	0/0	0/0	0/0	0/0
	5	2.21	2.29	Test Article	Erythema	0/0	0/0	0/0	0/0
					Oedema	0/0	0/0	0/0	0/0
				Negative Control	Erythema	0/0	0/0	0/0	0/0
					Oedema	0/0	0/0	0/0	0/0
	6	2.18	2.27	Test Article	Erythema	0/0	0/0	0/0	0/0
					Oedema	0/0	0/0	0/0	0/0
				Negative Control	Erythema	0/0	0/0	0/0	0/0
					Oedema	0/0	0/0	0/0	0/0
Primary irritation index						0			

Table 3 Positive control

Rabbit No	Group	Reaction	Interval (hours): score=left site/right site			
			1h	24h	48h	72h
1	Positive control	Erythema	0/0	1/2	2/3	3/3
		Oedema	0/0	2/1	2/2	3/3
	Negative Control	Erythema	0/0	0/0	0/0	0/0
		Oedema	0/0	0/0	0/0	0/0
2	Positive control	Erythema	0/1	2/1	3/3	4/3
		Oedema	1/0	2/2	3/3	3/4
	Negative Control	Erythema	0/0	0/0	0/0	0/0
		Oedema	0/0	0/0	0/0	0/0
3	Positive control	Erythema	1/0	1/2	3/3	4/3
		Oedema	0/1	2/1	3/4	3/4
	Negative Control	Erythema	0/0	0/0	0/0	0/0
		Oedema	0/0	0/0	0/0	0/0
Primary irritation index			5.2			

Positive control performed once every six months see CSTBB20020001P3(Finish date：2020-02-21)

191020340175

中国认可
国际互认
检测
TESTING
CNAS L13034

Skin Sensitization Test

Guinea Pig Maximization

Final Report

Article Name: Disposable surgical face mask

Report Number: CSTBB20030403

Method Standard: ISO 10993-10: 2010

Sponsor

Hunan EEXI Technology&Service Co.,Ltd.

No.6, North of Pingtou road, Liuyang Hi-tech industrial development zone, Hunan, China

Test Facility

CCIC Huatongwei international inspection (Suzhou) Co., Ltd

Room 101, Building G, Ruoshui Road 388, Suzhou, Jiangsu, China

CONTENTS

Notices

1. Please apply for rechecking within 15 days of receiving the report if there is any objection.
2. Any erasure or without special 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 report is only responsible for the test results of the tested samples.
5. The report shall not be reproduced except in full without the written approval of the company.

Abstract

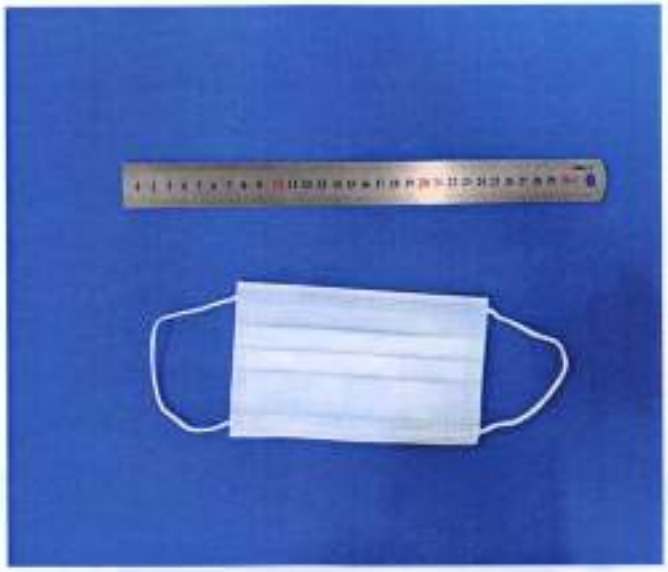
In this study, we took guinea pigs to observe the skin sensitization of the test article according to ISO 10993-10: 2010.

The test article were extracted in Constant Temperature Vibrator at 50 ℃, 60 rpm for 72 h by 0.9 % Sodium Chloride Injection and Sesame Oil. Mix 50:50 (by volume) stable emulsion of Freund's complete adjuvant with selected solvent. Intradermal induction and topical induction were operated in the clipped intrascapular region of each animal. After the topical induction phase was completed on day 14, all test and control animals were challenged with the test sample. The erythema and edema of the challenge site were observed to test the sensitization response of the test article. According to the Magnusson and Kligman scales, the response to erythema and edema at each application site of the skin was described and scored 24 hours and 48 hours after the challenge phase.

The results showed that the guinea pigs in the negative control group (0.9 % Sodium Chloride Injection, Sesame Oil) retained a normal appearance throughout the test and showed no skin irritants. A severe skin reactions for erythema and oedema were shown in the positive control group (DNCB). While in test article group, the response of skin on testing side did not exceed that on the control side. The skin reactions for erythema and oedema were not observed in test article group. The data of each group met the acceptance criteria, and the results of this test were considered valid.

Based on the above results, it can be concluded that under the experimental conditions, the test article Disposable surgical face mask has no potential skin sensitization on guinea pigs in the extraction method.

Study Verification and Signature



Protocol Number	SST2003015903BB
Protocol Effective Date	2020-03-23
Technical Initiation Date	2020-03-27
Technical Completion Date	2020-04-27
Final Report Completion Date	2020-05-11

Personnel _____ 2020-05-11
Date Completed

Approved _____ 2020-05-11
Study Director Date Completed

Supervisory _____
Test Facility Manager Date Completed

Huatongwei international inspection (Suzhou) Co., Ltd.

1.0 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.

2.0 Reference

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

Biological evaluation of medical devices-Part 12: Sample preparation and reference materials (ISO 10993-12:2012)

Biological evaluation of medical devices-Part 2: Animal welfare requirements (ISO 10993-2:2006)

3.0 Test and control articles

Groups	Test article	Negative Control Article(Polar)	Negative Control Article(Non-Polar)	Positive Control
Name	Disposable surgical face mask	Sodium Chloride Injection (SC)	Sesame Oil (SO)	2, 4-Dinitrochlorobenzene (DNCB)
Manufacture	Hunan EEXI Technology&Service Co.,Ltd.	Shijiazhuang No.4 Pharmaceutical	Jiangxi xinsen natural vegetable oil co., Ltd.	TOKYO CHEMICAL INDUSTRY CO., LTD
Size	17.5cm*9.5cm	500 ml	25 kg	25 g
Model	YX011	/	/	/
Lot Batch#	Not provided	1912121907	181120	H2UKD-DM
Test Article Material	Not provided	/	/	/
Physical State	Solid	Liquid	Liquid	Solid
Color	Not provided	Colorless	Light yellow	Light yellow
Package material	Not provided	/	/	/
Sterilization state	No	/	/	/
Concentration	/	0.9 %	/	Induction Concentration: 0.5 % Challenge Concentration: 1.0 % Dissolved in ethanol
Total Surface/Weight	Not provided	/	/	/
Storage Condition	Room Tep.	Room Tep.	Room Tep.	Room Tep.
The information about the test article was supplied by the sponsor wherever applicable.				

4.0 Identification of test system

4.1 Test animal

Species: Hartley Guinea Pig (Cavia Porcellus)

Number: 30 (20 Test +10 Control)

Sex: 15 ♀， 15 ♂

Initial body weight: 303.0~318.0 g

Health status: Healthy, not previously used in other experimental procedures

Animal identification: Ear tag

Cages: Plastic cage

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

4.2 Justification of test system

The albino guinea pig has been used historically for sensitization studies (Magnusson and Kligman, 1 970). The guinea pig is believed to be the most sensitive animal model for this type of study. DNCB is the positive control article recommended in the test instructions. To ensure the sensitivity of the experime ntal system, the positive control article should be verified every three months.

5.0 Animal Management

Animal purchase: Wuxi hengtai experimental animal breeding co. LTD SCXK (SU) 2015-0004

Bedding: Corncob Jiangsu Xietong Pharmaceutical Bio-engineering Co., Ltd.

Feed: Guinea pigs were fed with full-price pellets Jiangsu Xietong Pharmaceutical Bio-engineering Co., Ltd.

Water: Drinking water met the Standards for Drinking Water Quality GB 5749-2006

Animal room temperature: 18-26 ℃

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, or bedding expected to interfere with the test data.

6.0 Equipment and reagents

6.1 Instruments

Constant Temperature Vibrator (SHB007, calibration data: 2020/3/16), Autoclave (SHB026, calibration data: 2020/3/16), Electronic scale (SHB017, calibration data: 2020/3/16)

6.2 Reagents

Freund’s adjuvant Complete liquid (SIGMA, Lot No: SLBR3877V), Sodium dodecyl sulfate (SDS SIGMA, Lot No: SLBL2304V)

7.0 Experiment design

7.1 Sample preparation

The extracts of test article will be prepared according to the following steps:

Aseptic Sampling			Extraction in sterile vessels				
Sampling Manner	Actually sampling	Ratio	Reagent		Temperature	Time	pH
Whole	570.0 cm^2	6 cm^2: 1 ml	SC	95.0 ml	50 ℃	72 h	5.5
	570.0 cm^2		SO	95.0 ml			5.5

Both inducements and excitations were prepared by the number of times. The state of the leaching solution did not change visually after the leaching was advanced. After extraction, the samples were stored at room temperature for no more than 24 h. The extraction solution is clear, and the pH value has not been adjusted, filtered, centrifuged, diluted and other processes. The control solution was prepared under the same conditions.

7.2 Test method

7.2.1 Intradermal induction phaseI

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.

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 %); the control animals were injected with an emulsion of the blank liquid with adjuvant.

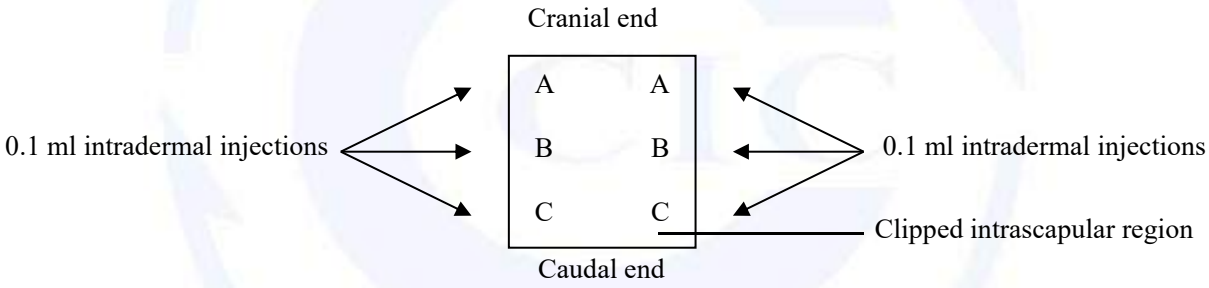


Figure 1 Location of intradermal injection sites

7.2.2 Topical induction phaseII

The maximum concentration that can be achieved in Intradermal induction phase Ⅰ did not produce irritation, animals are pretreated with 10% sodium dodecyl sulfate 24(±2) hours before the topical induction application.

At 7 d after completion of the intradermal induction phase, administer 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.

7.2.3 Challenge phase

At 14d after completion of the topical induction phase, challenge all test and control animals with the test sample. Absorbent gauzes (2.5 cmx2.5 cm) were soaked respectively with test article and 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.

8.0 The results observed

The day after challenge exposure, the patch will be removed and the area cleaned gently with gauze if necessary. The site will be wiped gently with a 0.9 % saline soaked gauze sponge prior to each scoring period. The challenge sites will be observed for signs of irritation and sensitization reaction, as indicated by erythema and edema. If necessary, the fur will be shaved or clipped in advance for the convenience of dermal score.

Daily challenge observation scores will be recorded approximately 24, and 48 hours after patch removal in accordance with the following classification system for skin reactions:

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/or swelling	3

9.0 Evaluation criteria

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.

10.0 Results of the test

All animals were survived and no abnormal signs were observed during the study.Individual results of dermal scoring for the challenge appear in Table 2.

11.0 Conclusion

The test article showed no evidence of causing delayed dermal contact sensitization in the guinea pig. Results and conclusions apply only to the test article tested. Any extrapolation of these data to other articles is the sponsor’s responsibility.

12.0 Record

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

13.0 Confidentiality Agreement

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

Table 2 Guinea pig Sensitization Dermal Reactions

Group		No.	Pretest weigh(g)	Finished weigh(g)	The Challenge patch was removed 24h later		The Challenge patch was removed 48h later		Positive rate
					Erythema	Swelling	Erythema	Swelling	
SC	Test	1	303.8	357.0	0	0	0	0	0%
		2	306.6	368.7	0	0	0	0	
		3	305.7	358.1	0	0	0	0	
		4	305.0	355.8	0	0	0	0	
		5	303.4	367.0	0	0	0	0	
		6	310.3	366.1	0	0	0	0	
		7	315.2	361.2	0	0	0	0	
		8	310.9	376.8	0	0	0	0	
		9	309.6	350.9	0	0	0	0	
		10	312.1	363.4	0	0	0	0	
	Control	11	312.7	353.9	0	0	0	0	—
		12	317.2	375.8	0	0	0	0	
		13	311.8	364.7	0	0	0	0	
		14	312.3	365.1	0	0	0	0	
		15	312.6	372.0	0	0	0	0	
SO	Test	16	318.0	379.5	0	0	0	0	0%
		17	306.6	366.8	0	0	0	0	
		18	311.3	361.1	0	0	0	0	
		19	310.7	378.4	0	0	0	0	
		20	309.1	379.0	0	0	0	0	
		21	304.9	362.1	0	0	0	0	
		22	311.0	364.8	0	0	0	0	
		23	303.2	376.8	0	0	0	0	
		24	307.4	364.6	0	0	0	0	
		25	303.2	352.5	0	0	0	0	
	Control	26	303.0	361.7	0	0	0	0	—
		27	312.6	370.6	0	0	0	0	
		28	303.7	360.4	0	0	0	0	
		29	309.3	351.5	0	0	0	0	
		30	310.2	366.1	0	0	0	0	

Table 3　Positive control

Group	No.	Pretest weigh(g)	Finished weigh(g)	The Challenge patch was removed 24 h later		The Challenge patch was removed 48 h later		Positive rate
				Erythema	Swelling	Erythema	Swelling	
Test	1	311.2	351.7	2	0	2	0	100%
	2	315.6	360.2	1	0	1	0	
	3	317.2	352.7	0	0	1	0	
	4	312.8	353.9	1	0	1	0	
	5	306.9	341.1	2	0	2	0	
	6	312.2	350.6	0	0	1	0	
	7	317.1	352.2	1	0	2	0	
	8	306.8	350.0	1	0	1	0	
	9	316.5	348.7	1	0	2	0	
	10	317.6	350.2	2	0	2	0	
Control	11	320.5	364.5	0	0	0	0	—
	12	307.6	350.2	0	0	0	0	
	13	306.9	345.1	0	0	0	0	
	14	310.0	352.3	0	0	0	0	
	15	315.8	346.6	0	0	0	0	

Note：The positive control was CSTBB20010001P1(Finish date：2020-02-07).

Ministero della Salute

Stampa | Scarica il dataset

Elenco dei dispositivi medici

Criteri di ricerca:
Denominazione fabbricante: **eexi**
Codice fiscale fabbricante:
Partita IVA / VAT number fabbricante:
Codice nazione fabbricante:
Denominazione mandatario:
Codice fiscale mandatario:
Partita IVA / VAT number mandatario:
Codice nazione mandatario:
Tipologia dispositivo:
Identificativo di registrazione attribuito dal sistema BD/RDM: **1989362**
Codice attribuito dal fabbricante:
Nome commerciale e modello:
Classificazione CND:
Descrizione CND:
Classe CE (valida solo per dispositivi medici di classe, impiantabili attivi e IVD):

Elenco dispositivi individuati

Dati aggiornati al:20/04/2021

DISPOSITIVO MEDICO/ASSEMBLATO									FABBRICANTE/ASSEMBLATORE				
TIPOLOGIA DISPOSITIVO	IDENTIFICATIVO DI REGISTRAZIONE BD/RDM	ISCRITTO AL REPERTORIO	CODICE ATTRIBUITO DAL FABBRICANTE/ASSEMBLATORE	NOME COMMERCIALE E MODELLO	CND	CLASSE CE	DATA PRIMA PUBBLICAZIONE	DATA FINE IMMISSIONE IN COMMERCIO	RUOLO AZIENDA	DENOMINAZIONE	CODICE FISCALE	PARTITA IVA/VAT NUMBER	NAZIONE
Dispositivo	1989362	S	YX011	MASCHERINA CHIRURGICA IIR	T020601 - MASCHERINE CHIRURGICHE STANDARD	I - Classe I non sterile e senza funzioni di misura	01/09/2020		FABBRICANTE	HUNAN EEXI TECHNOLOGY & SERVICE CO., LTD.			CN
									MANDATARIO	SHANGHAI INTERNATIONAL HOLDING CORP. GMBH (EUROPE)		00166892350	DE

<< < Pagina:1 > >> Num. Pagine:1 Num. Dispositivi:1

SCHEDA TECNICA MASCHERINA CHIRURGIGA TIPO IIR CON ELASTICI CODICE: YX011

Registrazione Ministero della Salute repertorio No. **CND T020601 - RDM 1989362**

Descrizione prodotto:

Mascherina chirurgica monouso a tre starti, in tessuto non tessuto TNT in polipropilene PP opaco, inodore, con filtro ad elevata efficienza, priva di fibra di vetro, ipoallergenico, con ottima permeabilità all'aria. La mascherina è dotata di elastici privi di lattice. Sistema di rifinitura termosaldata.

Il **Prodotto è monouso ed altamente professionale con elevato potere filtrante maggiore del 98%,**

la particolarità costruttiva rende la mascherina particolarmente confortevole per l'uso professionale e proteggono dalla contaminazione di naso e bocca e dalla inalazione di particelle di dimensioni inferiori al micron aero disperse; Inoltre la loro bassissima resistenza permette di indossarla per molto tempo.

Le mascherine, come tutti gli indumenti monouso devono essere indossati una sola volta chiaramente si raccomanda dopo l'uso di gettarle nel contenitore per rifiuti speciali.
Dopo la rimozione della mascherina eseguire sempre l'igiene delle mani lavaggio con sapone antisettico specifico.
Le mascherine devono essere personali e utilizzate al massimo per la durata di un turno lavorativo.
Inoltre devono comunque essere sostituite immediatamente quando risultano danneggiate, o visibilmente contaminate

Normative di riferimento:

Direttiva CEE 93/42, Direttiva 47/2007
Mascherina chirurgica tipo IIR/EN14683

CODICE PRODOTTO: YX011 Mascherina Chirurgica tipo IIR
Produttore: : Hunan EEXI Technology & Service Co.,Ltd. No.6, North of Pingtou road, Liuyang Hi-tech industrial development zone, Hunan, China.
Luogo di Produzione: No.6, North of Pingtou road, Liuyang Hi-tech industrial development zone, Hunan, China.

Mascherina chirurgica tipo IIR CODICE. YX011	
MISURA	UNICA
DIMENSIONI	17,5 cm x 9,5 cm.

Il prodotto è classificato CLASSE I-Direttiva 93/42 CEE – Direttiva n. 47/2007

Le mascherine chirurgiche tipo II R YX 011 sono realizzate con le seguenti caratteristiche identificative:

- Monouso
- Con elastici
- Tipo IIR
- A tre strati
- Latex free
- Atossica, conformabile
- Stringinaso modellabile nichel free
- Assenza fibre di vetro
- Non irritante
- TNT tipo (SMS)
- La mascherina chirurgica crea una barriera fisico meccanica eludendo il passaggio di agenti infettivi rilasciati dalla bocca e dal naso dell'utilizzatore verso l'esterno. La mascherina impedisce il passaggio di essudati mantenendo la permeabilità dell'aria.
- Colore : Azzurro (verso l'esterno), bianco (verso l'interno)

- Capacità filtrante: > 98%

La mascherina tipo I Cod.YX011 è conforme alle seguenti norme:

- UNI EN 14683:2019
- UNI ISO 10993:2010

Dimensioni e tolleranze +/- 5%

Materiale filtrante n. 3 strati di SMS – Thermobonded Nonwoven Hydrophobic 100% polipropilene
Resistenza allo strappo come da test effettuati
Nasello: Incapsulato extra – leggero per la conformazione al viso
Elastici: atraumatici in cotone resistente anallergico
La mascherina è interamente saldata ad ultrasuoni, non contiene collanti, agenti chimici o resine, totale assenza di fibre di vetro e completamente Latex free, è idrorepellente esternamente.
L'articolo qui descritto non è sterile, la sterilizzazione può essere eseguita su richiesta del Committente.

Confezionamento: Confezioni standard in buste da Pz.10, le buste a loro volta saranno inserite in una scatola contenente n.50Pz. (5 buste). L'imballo nel cartone sarà composto da n. 40 scatole per un totale di 2.000 Pz. Dimensioni imballo:52x40x37cm/0,0762 mc-9,1 Kg/cartone.

Le mascherine chirurgiche della serie YX hanno lo scopo di **evitare che chi le indossa contamini l'ambiente**, in quanto limitano la trasmissione di agenti infettivi e ricadono nell'ambito dei dispositivi medici di cui a Direttiva. 24 febbraio 1997, n.46 e s.m.i..

Possono essere utilizzate in ambiente ospedaliero e in luoghi ove si presti assistenza a pazienti (ad esempio case della salute, ambulatori, ecc).

Test Report

(Electronic version)

Verification Website: www.gttc.net.cn

Verification Code: TLNK-6353-44

No:20R000012MO Issue Date: 2020-04-24

Applicant: HUNAN EEXI TECHNOLOGY&SERVICE CO.,LTD.
Address: NO.6, NORTH OF PINGTOU ROAD, LIUYANG HI-TECH INDUSTRIAL DEVELOPMENT ZONE, HUNAN, CHINA

Information confirmed by applicant:

Disposable surgical mask(non-sterile)

Quantity: one hundred pieces

Model: YX011 YX109

Classification: Type Ⅱ R

Standard Adopted:

EN 14683:2019+AC:2019 <Medical face masks-Requirements and test methods>

Date Received/Date Test Started: 2020-03-30

Conclusion:

Bacterial filtration efficiency (BFE)	M
Microbial cleanliness	M
Differential pressure	M
Splash resistance pressure	M

Note: "M"-Meet the standard's requirement "F"-Fail to meet the standard's requirement "---"-No comment

Remark:
The authorization of bacterial filtration efficiency (BFE), differential pressure, splash resistance pressure is not received from CNAS.
MODIFIED CONTENT: MODIFIED CLIENT CONFIRMED INFORMATION.
THIS REPORT REPLACES TEST REPORT 20R000012 WHICH HAS BECOME INVALID AUTOMATICALLY.
All the tested items are tested under the standard condition (except for indication).
Copies of the report are valid only re-stamped.
The experiment was carried out at No.1, Zhujiang Road, Panyu District, Guangzhou, Guangdong, P.R.China.

Approved By: Nan Ma

Nan Ma Engineer

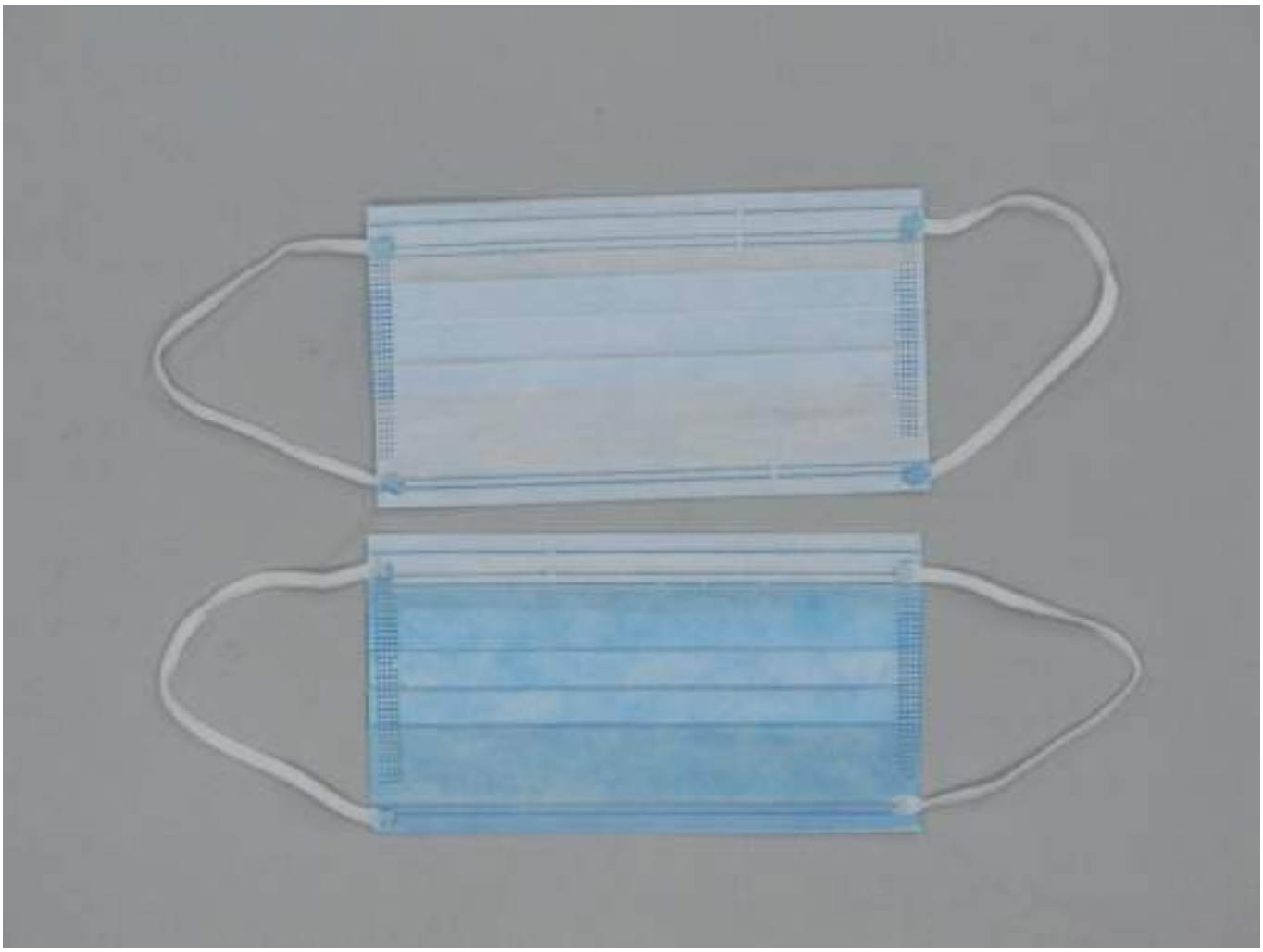
Guangzhou Inspection Testing and Certification Group Co.,Ltd. Inspection & Testing services

Guangzhou Inspection Testing and Certification Group Co.,Ltd.
Add: No.1, Zhujiang Road, Panyu District, Guangzhou, Guangdong, P.R.China.
Tel: +86-20-61994598 61994599

Test Report

(Electronic version)

No: 20R000012MO



Guangzhou Inspection Testing and Certification Group Co.,Ltd.
Add: No.1, Zhujiang Road, Panyu District, Guangzhou, Guangdong, P.R.China.
Tel: +86-20-61994598 61994599

Test Report

(Electronic version)

No: 20R000012MO

Bacterial filtration efficiency (BFE)
Test method: EN 14683: 2019+AC: 2019 Annex B

Test principle:
A specimen of the mask material is clamped between a six-stage cascade impactor and an aerosol chamber. An aerosol of Staphylococcus aureus is introduced into the aerosol chamber and drawn through the mask material and the impactor under vacuum. The bacterial filtration efficiency (BFE) of the mask is given by the number of colony forming units passing through the medical face mask material expressed as a percentage of the number of colony forming units present in the challenge aerosol.

Test equipment:
Incubator
Electronic balance
Autoclave
Experimental system for bacterial filtration efficiency (BFE) of mask

The environmental conditions of the laboratory and test condition:
Total bacteria: 0 CFU/plate
Total fungi: 0 CFU/plate
Blank experiment: Aseptic growth
Test environment temperature: 24.5℃, Relative humidity: 56.0%
Culture medium: TSA agar medium
Culture temperature: 37℃， Culture time: 48h
Test bacteria : staphylococcus aureus ATCC 6538
Concentration of bacterium: 5.0×10^{5} CFU /ml
Positive control average (C): 1.9×10^{3} CFU
Negative monitor count: ＜1 CFU
Test area: 40 cm^{2}
Flow rate: 28.3 l/min
Pretreatment: Condition each specimen for 4 h by exposure to a temperature of (21±5) ℃ and a relative humidity of (85±5)%
Mean particle size: 3.0 μm
The medical face mask in contact with the bacterial challenge: inside

Guangzhou Inspection Testing and Certification Group Co.,Ltd. Inspection & Testing services

Guangzhou Inspection Testing and Certification Group Co.,Ltd.
Add: No.1, Zhujiang Road, Panyu District, Guangzhou, Guangdong, P.R.China.
Tel: +86-20-61994598 61994599

Test Report

(Electronic version)

No: 20R000012MO

Results:

Sample	T	BFE (%)	Requirement (%)	Classification	Conclusion
1	21	98.89	≥98 EN 14683:2019+AC:2019	Type ⅡR	Pass
2	25	98.68			
3	21	98.89			
4	21	98.89			
5	24	98.74			

Remarks:

For each test specimen calculate the bacterial filtration efficiency B, as a percentage, using the following formula:

$B = (C - T) / C \times 100$

where

B is bacterial filtration efficiency (BFE), %;

C is positive control average;

T is the total plate count for the test specimen.

Guangzhou Inspection Testing and Certification Group Co.,Ltd. Inspection & Testing services

Guangzhou Inspection Testing and Certification Group Co.,Ltd.
Add: No.1, Zhujiang Road, Panyu District, Guangzhou, Guangdong, P.R.China.
Tel: +86-20-61994598 61994599

Test Report

(Electronic version)

No: 20R000012MO

Microbial cleanliness
Test method: EN ISO 11737-1:2018, Membrane filtration

Test principle:
Take the required samples from the original packaging. Weigh a certain amount of sample and placed in a sterile 500 ml bottle containing 300 ml of extraction liquid (1 g/l Peptone, 5 g/l NaCl and 2 g/l Tween 20). The bottle is laid down on an orbital shaker and shaken for 5 min at 250 rpm. After this extraction step, 100 ml of the extraction liquid is filtered through a 0.45 µm filter and laid down on a TSA plate for the total viable aerobic microbial count. Another 100 ml aliquot of the same extraction liquid is filtered in the same way and the filter plated on Sabouraud Dextrose agar (SDA) for fungi enumeration. The plates are incubated for 3 days at 30℃ and 7 days at (20 to 25)℃ for TSA and SDA plates respectively. The total bioburden is expressed by addition of the TSA and SDA counts.

Test equipment:
Constant temperature incubator
Electronic balance
Pressure steam sterilizer
Biosafety cabinet

The environmental conditions of the laboratory and test condition:
Test environment temperature: 24.5℃, Relative humidity: 56.0%
Test environment monitoring: total bacteria: 0 CFU/plate, total fungi: 0 CFU/plate, blank experiment: aseptic growth

Guangzhou Inspection Testing and Certification Group Co.,Ltd. Inspection & Testing services

Guangzhou Inspection Testing and Certification Group Co.,Ltd.
Add: No.1, Zhujiang Road, Panyu District, Guangzhou, Guangdong, P.R.China.
Tel: +86-20-61994598 61994599

Test Report

(Electronic version)

No: 20R000012MO

Results:

Microbial	Measured value (CFU/g)	Microbial cleanliness (CFU/g)	Requirement (CFU/g)	Classification	Conclusion
Bacteria	3	3	≤30 EN 14683:2019+AC:2019	**Type ⅡR**	**Pass**
Fungi	0				

Guangzhou Inspection Testing and Certification Group Co.,Ltd.
Add: No.1, Zhujiang Road, Panyu District, Guangzhou, Guangdong, P.R.China.
Tel: +86-20-61994598 61994599

Test Report

(Electronic version)

No: 20R000012MO

Differential pressure

Test method: EN 14683:2019+AC:2019 Annex C

Test principle:

This procedure was performed to evaluate the differential pressure of the medical face mask material by measuring the air exchange pressure through a measured surface area at a constant air flow rate.

Test equipment:

GTTC-YLC-1 Apparatus for measuring differential pressure

The environmental conditions of the laboratory and test condition:

Air flow: 8 l/min

Test area: 4.9cm^2

Pretreatment: Condition each specimen for a minimum of 4 h by exposure to a temperature of (21±5) ℃ and a relative humidity of (85±5)%

General location of the areas of the mask the differential measurements: specimen center

Guangzhou Inspection Testing and Certification Group Co.,Ltd. Inspection & Testing services

Guangzhou Inspection Testing and Certification Group Co.,Ltd.
Add: No.1, Zhujiang Road, Panyu District, Guangzhou, Guangdong, P.R.China.
Tel: +86-20-61994598 61994599

Test Report

(Electronic version)

No: 20R000012MO

Results:

Sample	Measured value (Pa)	Differential pressure (Pa/cm²)	Requirement (Pa/cm²)	Classification	Conclusion
1	169	34.3	＜60 EN 14683:2019+AC:2019	**Type ⅡR**	**Pass**
2	163				
3	174				
4	170				
5	166				
Average	168				

Guangzhou Inspection Testing and Certification Group Co.,Ltd. Inspection & Testing services

Guangzhou Inspection Testing and Certification Group Co.,Ltd.
Add: No.1, Zhujiang Road, Panyu District, Guangzhou, Guangdong, P.R.China.
Tel: +86-20-61994598 61994599

Test Report

(Electronic version)

No: 20R000012MO

Splash resistance pressure
Test method: ISO 22609:2004

Test principle:
A specimen medical face mask is supported on an apparatus. A volume of synthetic blood is sprayed horizontally at the specimen mask to simulate the scenario of a mask being splashed by a punctured blood vessel. The volume of fluid, distance to impact, orifice size and fluid velocity are defined in this method and intended to be consistent with this health care scenario. Any evidence of synthetic blood penetration on the side of the medical face mask contacting the wearer's face constitutes failure. Results are reported as "pass/fail". Specimen medical face masks are evaluated at a total of three different velocities corresponding to human blood pressures of 10.6 kPa, 16.0 kPa, and 21.3 kPa. Test results are reported at each velocity and the medical face mask is rated at the highest corresponding blood pressure for which medical face mask specimens demonstrate an acceptable quality limit of 4.0.

Test equipment:
Test apparatus for synthetic blood penetration LFY-227
Air compressor
Graduated cylinder
Electronic balance
Targeting plate

The environmental conditions of the laboratory and test condition:
Pretreatment: Condition each specimen for 24 h by exposure to a temperature of (21±5) ℃ and a relative humidity of (85±5)%
Surface tension of synthetic blood: 0.042 N/m
Pressure: 16.0 kPa
Velocity: 550 cm/s

Guangzhou Inspection Testing and Certification Group Co.,Ltd. Inspection & Testing services

Guangzhou Inspection Testing and Certification Group Co.,Ltd.
Add: No.1, Zhujiang Road, Panyu District, Guangzhou, Guangdong, P.R.China.
Tel: +86-20-61994598 61994599

Test Report

(Electronic version)

No: 20R000012MO

Results:

Sample	Measured value	Requirement (kPa)	Classification	Conclusion
	Pressure			
	16.0 kPa			
1	pass	≥16.0 EN 14683:2019+AC:2019	Type ⅡR	Pass
2	pass			
3	pass			
4	pass			
5	pass			
6	pass			
7	pass			
8	pass			
9	pass			
10	pass			
11	pass			
12	pass			
13	pass			
14	pass			
15	pass			
16	pass			
17	pass			
18	pass			
19	pass			
20	pass			
21	pass			
22	pass			
23	pass			
24	pass			
25	pass			
26	pass			
27	pass			
28	pass			
29	pass			
30	pass			
31	pass			
32	pass			
Final result	pass			

Remarks:

An acceptable quality limit of 4.0 % is met for a single sampling plan when 29 or more of the 32 tested specimens show "pass" results.

Guangzhou Inspection Testing and Certification Group Co.,Ltd. Inspection & Testing services

———End of Report———

Guangzhou Inspection Testing and Certification Group Co.,Ltd.
Add: No.1, Zhujiang Road, Panyu District, Guangzhou, Guangdong, P.R.China.
Tel: +86-20-61994598 61994599

Declaration of Conformity

Manufacturer: Hunan EEXI Technology&Service Co.,Ltd.

Address: No.6, North of Pingtou road, Liuyang Hi-tech industrial development zone, Hunan, China

EU Authorised Representative:
Shanghai International Holding Corp. GmbH (Europe)

Address: Eiffestrasse 80, 20537 Hamburg, Germany

DIMDI code: DE/0000040627

Registration number: DE/CA05/MP-238321-2557-00

Device: Disposable surgical face mask

Type: Type IIR **Model:** YX011

Classification (93/42/ECC Annex IX Rules 1): Class I

Conformity assessment route: Annex VII

We, Hunan EEXI Technology&Service Co.,Ltd. herewith declare on our exclusive responsibility that the above mentioned products meet the provisions of the Council Directive 93/42/EEC and 2007/47/EC for medical devices as transposed into national law. All supporting documentation is retained under the premises of the manufacturer.

CE

Standard Applied:

Standards	Standard's title
ISO 13485:2016	Medical Device - Quality management systems - Requirement for regulatory purposes
EN ISO 14971:2019	Medical devices - Application of risk management to medical devices
EN ISO15223-1:2016	Medical devices - Symbols to be used with medical device lables, labelling and information to be supplies – Part 1: General requirements
EN 1041:2008	Information supplied by the manufacturer of medical devices
MEDDEV 2.7.1:REV4.	Evaluation Of Clinical Data : A Guide For Manufacturers and Notified Bodies
EN 14683:2019	Medical face masks - Requirements and test methods
EN ISO 10993-1:2009	Biological evaluation of medical devices – Part 1: Evaluation and testing within a risk management process
EN ISO 10993-5:2009	Biological evaluation of medical devices-Part 5: Test for In Vitro Cytotoxicity.
EN ISO 10993-10:2013	Biological evaluation of medical devices-Part 10: Tests for irritation and skin sensitization

Date of Issue:

03rd April 2020

Signature:

Robbin Liu

General Manager

191020340175

中国认可
国际互认
检测
TESTING
CNAS L13034

In Vitro Cytotoxicity Test

MTT Method

Final Report

Article Name: Disposable surgical face mask

Report Number: CSTBB20030396

Method Standard: ISO 10993-5：2009

Sponsor

Hunan EEXI Technology&Service Co.,Ltd.

No.6, North of Pingtou road, Liuyang Hi-tech industrial development zone, Hunan, China

Test Facility

CCIC Huatongwei international inspection (Suzhou) Co., Ltd

Room 101, Building G, Ruoshui Road 388, Suzhou, Jiangsu, China

CONTENTS

Notices

1. Please apply for rechecking within 15 days of receiving the report if there is any objection.
2. Any erasure or without special 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 report is only responsible for the test results of the tested samples.
5. The report shall not be reproduced except in full without the written approval of the company.

Abstract

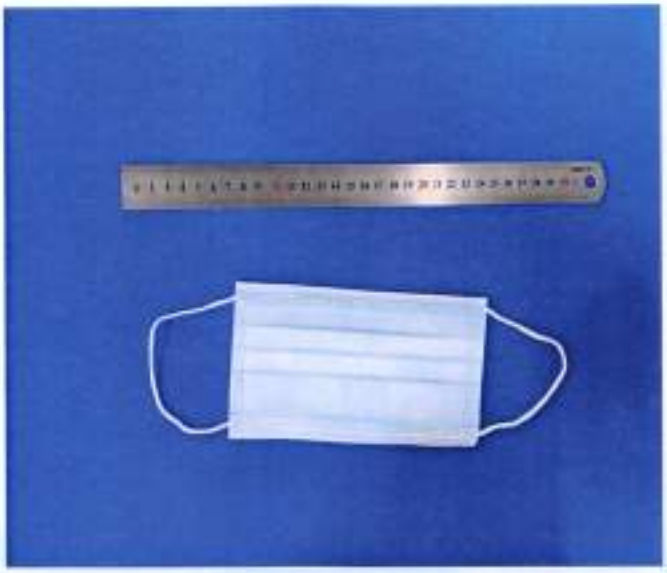
In this study, mammalian L-929 cells were cultured in vitro according to ISO 10993-5:2009 to test the potential cytotoxicity of the test article.

The test articles and the control material were separately placed in MEM medium containing 10% fetal bovine serum, and extracted in a 37 ℃ incubator for 24 hours. After the end of the extraction, the cell culture medium in the 96-well plate (10^4 cells/well) cultured for 24 hours was removed and replaced with the corresponding extract, cultured in 37 ℃, 5% CO_2, >90% humidity for 24 hours. After the culture, the morphology and cell lysis of the cells were observed under the microscope, and the cytotoxicity of the test samples was determined by MTT assay.

The results showed that the cells in the blank control group and the negative control group (high density polyethylene) were well-formed throughout the experiment and showed no cytotoxic reaction. A severe cytotoxic response was shown in the positive control group (ZDEC). The 100% concentration of the test extract retained a normal appearance after 24 hours of incubation, and the cell viability was 76.6%. The data of each group met the acceptance criteria, and the results of this test were valid.

Based on the above results, it can be concluded that under the experimental conditions, the test article Disposable surgical face mask have no potential toxicity to L-929 in the MTT method.

Study Verification and Signature



Protocol Number	SST2003015901BB
Protocol Effective Date	2020-03-23
Technical Initiation Date	2020-03-23
Technical Completion Date	2020-03-25
Final Report Completion Date	2020-05-11

Personnel _____ 2020-05-11
Date Completed

Approved
Study Director _____ 2020-05-11
Date Completed

Supervisory
Test Facility Manager _____ Date Completed

Huatongwei international inspection (Suzhou) Co., Ltd.

1.0 Purpose

The purpose of the test is to determine the potential cytotoxicity toxicity of a mammalian cell culture (mouse fibroblast L-929 cells) in response to the test article.

2.0 Reference

Biological evaluation of medical devices-Part 5: Tests for In Vitro Cytotoxicity (ISO 10993-5：2009)

Biological evaluation of medical devices-Part 12: Sample preparation and reference materials (ISO 10993-12：2012)

3.0 Test and control articles

Groups	Test article	Negative Control Article	Positive Control Article	Blank Control
Name	Disposable surgical face mask	High Density Polyethylene Film	ZDEC	MEM medium, with addition 10% FBS
Manufacture	Hunan EEXI Technology&Service Co.,Ltd.	Hatano Research Institute. FDSC	Sigma-Aldrich.	Hyclone
Size	17.5cm*9.5cm	3 cm×10 cm (5 sheets)	25 g	500 ml
Model	YX011	/	/	/
Lot Batch#	Not provided	C-161	BCBQ6847V	AD23420275
Test Article Material	Not provided	/	/	/
Physical State	Solid	Solid	Solid	Liquid
Color	Not provided	White	White	Pink
Packaging Material	Not provided	/	/	/
Sterilized or Not	No	No	No	Yes
Concentration	/	/	0.1%	/
Total Surface	Not provided	/	/	/
Storage Condition	Room Tep.	Room Tep.	Room Tep.	4℃
Note：The information about the test article was supplied by the sponsor wherever applicable.				

4.0 Identification and justification of test system

L-929 mouse fibroblast cells obtained from American Type Culture Collection (ATCC).

L-929 cells have been used for cytotoxicity studies because they demonstrate sensitivity to extractable cytotoxic articles. Also, the test article is extracted and administered in vitro to mouse fibroblast L929 cells through a solvent compatible with the test system, which is the optimal route of administration available in this test system as recommended in ISO 10993-5.

5.0 Equipment and reagents

5.1 Instruments

Vertical pressure steam sterilizer (SHB026), CO_2 Incubator (SHB002), Steel Straight Scale (SHB076), Electronic Balance (SHB016), Clean bench (SHB014), Multiskan Spectrum Microplate Spectrophotometer (SHB003), Bench type low speed centrifuge (SHB022), Inverted microscope (SHB005)

5.2 Reagents

MEM (Hyclone, AD23420275), FBS (Clark, JC65113), Penicillin-Streptomycin (Gibco, 15140122), Try psin (Gibco, 25200056), PBS (Hyclone, AD21380277), MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyletrazo lium bromide) (Sigma, MKBG20386), Isopropyl alcoho (Macklin, C10394867)

6.0 Experiment design and dose

6.1 Sample preparation

According to the table below, aseptic extraction of the test article sealed and incubated in MEM medium (10% FBS) at 37 ℃, 5% CO_2 and 60 rpm for 24 hours.

Groups	Sampling		Sterilization	Aseptic Extraction In Inert Container				Final Extract
	Sampling Manner	Actually sampling	Method	Ratio	Extracts	Condition	pH	Clear or Not
Test article	Whole	570.0 cm^2	EO	6 cm^2: 1 ml	95.0 ml	37 ℃ 24 h	7.4	Clear
Negative Control	Random	60.0 cm^2	UV	3 cm^2: 1 ml	20.0 ml	37 ℃ 24 h	7.4	Clear
Positive Control	Random	0.02 g	Filter	0.1 g: 100 ml	20.0 ml	37 ℃ 24 h	7.4	Clear
Blank Control	/	/	/	/	10.0 ml	37 ℃ 24 h	7.4	Clear

The changes of the leaching solution was observed after extraction. No particulates or color changes were observed in pre- and post-extraction, and immediately be used in the follow-up experiment. The color and pH of the extraction solution did not change before and after use, and the pH value was 7.4 after leaching.

6.2 Test method

Aseptic procedures were used for handling cell cultures. L-929 cells were cultured in MEM medium (10% FBS, 1% Penicillin-Streptomycin solution) at 37 ℃ in a humidified atmosphere of 5% CO_2, then digested by 0.25% trypsin containing EDTA to get single cell suspension. 1×10^5 cells/ml suspension were obtained by centrifuging (1000 rpm, 5 min) and re-dispersing in MEM medium.

The suspended cells were dispensed at 100 µl per well in 96-well plate, and cultured in cell incubator (5% CO_2, 37 ℃, >90% humidity). Cell morphology was evaluated to verify that the monolayer was satisfactory.

After the cells grew to about 70% and form a monolayer, original culture medium was discarded. The 96-well plates were then treated with 100 µl of extract of test article (100%、75%、50%、25%), control article, negative article and positive article respectively. The 96-well plate was incubated at 37 ℃ in cell incubator of 5% CO_2 for 24 h. Six replicates of each test were tested.

After incubation, observe the cell morphology first and then discard the culture medium. Add 50 µl MTT (1mg/ml) to each well and then incubated at 37 ℃ in a humidified atmosphere of 5% CO_2 for 2 hours. The liquid in

each well was tipped out and 100 µl Isopropyl alcoho was added to each well to suspend the cell layer.

Evaluate the suspension above with a dual-wavelength spectrophotometer with the measurement wavelength at 570 nm.

7.0 Statistical method

Mean±standard deviation ($\bar{x}$±s)

The cell cytotoxicity ratio = OD_{570} of test (or positive or negative) article group/ OD_{570} of blank control group×100%.

8.0 Evaluation criteria

8.1 The 50% extract of the test article should have at least the same or a higher viability than the 100% extract. Otherwise the test should be repeated.

8.2 The lower the Viab.% value, the higher the cytotoxic potential of the test article is.

8.3 If viability is reduced to < 70% of the blank, it has a cytotoxic potential.

8.4 The Viab.% of the 100% extract of the test article is the final result.

9.0 Results of the test

9.1 Results of the cell morphology

Table 1 Observation of the cell morphology

Group	Before inoculation	Before treated with extract	24 h after treatment
Blank control	Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth.	Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth.	Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth.
Negative control			Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth.
Positive control			Nearly complete or complete destruction of the cell layers.
100% Test article extract			The cells showed a round shape and a change in cell morphology occasionally, and there were particles in the cytoplasm, occasionally cell lysis and slight growth inhibition.
75% Test article extract			The cells showed a round shape and a change in cell morphology occasionally, and there were particles in the cytoplasm, occasionally cell lysis and slight growth inhibition.
50% Test article extract			The cells showed a round shape and a change in cell morphology occasionally, and there were particles in the cytoplasm, occasionally cell lysis and slight growth inhibition.
25% Test article			The cells showed a round shape and a change

extract			in cell morphology occasionally, and there were particles in the cytoplasm, occasionally cell lysis and slight growth inhibition.

9.2 Results of the cell vitality

Table2 Results of the cell vitality

Group	OD value								Viab. (%)
	1	2	3	4	5	6	$\bar{x}$	s	
Blank control	0.601	0.663	0.638	0.655	0.606	0.629	0.632	0.025	100.0
Negative control	0.598	0.582	0.598	0.599	0.597	0.593	0.595	0.006	94.1
Positive control	0.054	0.062	0.061	0.059	0.068	0.064	0.061	0.005	9.7
100% test article extract	0.477	0.471	0.481	0.491	0.472	0.511	0.484	0.015	76.6
75% test article extract	0.512	0.508	0.485	0.494	0.514	0.498	0.502	0.011	79.4
50% test article extract	0.514	0.511	0.516	0.512	0.505	0.501	0.510	0.006	80.7
25% test article extract	0.521	0.535	0.525	0.518	0.524	0.538	0.527	0.008	83.4

10.0 Conclusion

Under the conditions of this study, the test article have no potential toxicity to L-929 cells.

11.0 Record

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

12.0 Confidentiality Agreement

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