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检测
TESTING
CNAS L2954

苏州大学
卫生与环境技术研究所
最终报告

报告编号: SDWH-M201801609-1

参照 ISO 10993-5: 2009 方法进行
医疗卫生用非织造布的细胞毒性试验
浸提法
含 10%胎牛血清的 MEM 浸提液

委托单位
常州锦欣达纤维新材料有限公司
制造商
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目 录

检测报告说明

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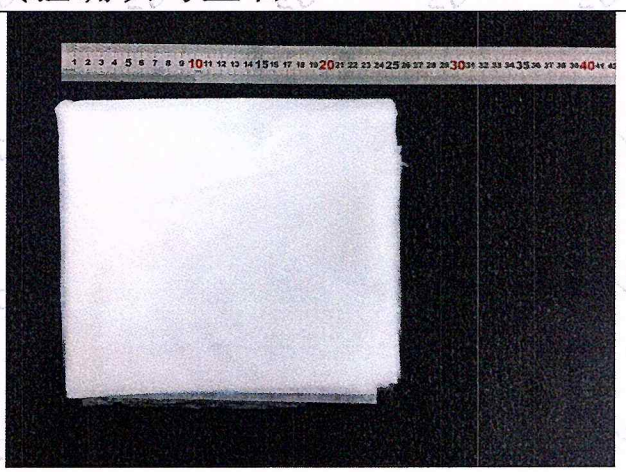
试验确认与签名

试验样品	
接样日期：	2018-05-30
试验计划书编号：	SDWH-PROTOCOL-GLP-M201801609-1
试验计划书生效日期：	2018-06-11
试验操作开始日期：	2018-06-12
试验操作结束日期：	2018-06-22
报告完成日期：	2018-07-02

编制：王德衡　　2018.07.02
日期

审核：朱雨婷　　2018.07.02
试验负责人　　日期

签发：　　2018-07-02
授权签字人　　日期

苏州大学卫生与环境技术研究所

质量控制声明

试验过程恪守美国食品药品监督局《非临床研究实验室的良好实验室规范》21 CFR 58 部分。

豁免执行的条款为：21 CFR 条款 58.105 和 58.113，样品鉴定和样品与载体的混合物的稳定性。

质量办公室负责监督试验过程，监督日期见下表，并报告试验负责人和 SDWH 管理层。

监督	监督日期	数据报告试验负责人	数据报告 SDWH 管理层
试验过程	2018-06-19	2018-06-19	2018-07-02
原始数据	2018-07-02	2018-07-02	2018-07-02
报告	2018-07-02	2018-07-02	2018-07-02

质量办公室：_____ 2018-07-02

QA　　日期

1.0 摘　要

试验样品浸提液与生长旺盛的 L929 细胞培养（37℃ ,5% CO_2）48h 后，观察细胞形态，细胞裂解情况。结果显示供试品组为 0 级，对照组结果显示本次试验结果有效。

在本次试验条件下，供试品医疗卫生用非织造布对 L929 细胞无毒性影响。

2.0 目　的

该试验目的是为了评价试验样品对 L929 哺乳动物成纤维细胞的生物学反应。

3.0 参考标准

医疗器械的生物学评价-第 5 部分：细胞毒性测试-体外法 ISO 10993-5：2009
医疗器械的生物学评价-第 12 部分：样品制备和参照样品 ISO 10993-12：2012

4.0 执行规范

美国食品药品监督局《非临床研究实验室的良好实验室规范》21 CFR 58 部分

ISO/IEC 17025:2005《检测和校准实验室能力的通用要求》CNAS-CL01 检测和校准实验室能力认可准则（中国合格评定国家认可委员会实验室认可证书 No.CNAS L2954）

检验检测机构资质认定评审准则 (中国国家认证认可监督管理委员会 资质认定 中国检验机构和实验室强制性批准 CMA 180015144061)

5.0 对照和试验样品确定

5.1 试验样品名称：医疗卫生用非织造布
来样原始状态：未灭菌
CAS 编号：未提供
型号：未提供
规格：双 S 亲水无纺布
批号：JXD2018005
样品材料：无纺布
包装材质：未提供
性状：固体
颜色：白色
密度：未提供
稳定性：未提供
溶解度：未提供
保存条件：室温
以上试验样品信息是由样品委托单位提供。委托单位负责遵守 GLP 试验有关样品的相关规定。

5.2 阴性对照
名称：高密度聚乙烯
制造商：美国药典委员会

规格： 3 片装
批号：K0M357
性状：固体
颜色：白色
稳定性：室温下稳定
保存条件：室温

5.3 阳性对照样品名称：Zinc diethyldithiocarbamate
制造商：Sigma
规格：25g
批号：MKBD516V
浓度：1%
溶剂：10%胎牛血清的 MEM 培养液
配制日期：2018-06-19
性状：固体
颜色：白色
保存条件：4±2 ℃

6.0 试验系统鉴别

该试验用小鼠成纤维细胞 L929,细胞系来自美国菌种保存中心。

7.0 试验系统确认

小鼠成纤维细胞 L929 用来检测细胞毒性试验是因为其细胞毒性反应灵敏。

8.0 给药途径确认

细胞毒性体外法已经用于医疗器械生物相容性细胞毒性的评价。

9.0 试验设计

9.1 试验和对照样品制备

无菌操作按下表的比例（样品：浸提液体积）用含 10%胎牛血清的 MEM 培养液浸提样品，于 37℃浸提 24 小时。浸提前后浸提液状态未发生改变。浸提液立即用于实验。浸提液 pH 值未经调整，未经过滤，离心，稀释等处理过程。

无菌操作取样		灭菌方式	惰性容器内无菌浸提			最终浸提液	
取样方式	实际取样	钴 60 25kGy	取样比例	浸提液	条件	pH	是否澄清
随机取样	120cm²		6cm² : 1ml	20.0ml	37℃，24h	7.4	澄清

同法制备阴性对照样品和阳性对照样品。

9.2 仪器设备

高压灭菌器（SDWH2204）校正有效期（2019-05-15）
恒温摇床（SDWH2109） 校正有效期（2018-11-12）
CO_2 培养箱（SDWH021）校正有效期（2019-05-15）
倒置显微镜（SDWH037）校正有效期（2018-08-28）
超净工作台（SDWH454）校正有效期（2019-05-20）
电子天平（SDWH056）校正有效期（2019-01-21）
冰箱（SDWH-448）

9.3 试剂

胎牛血清（CORNING，批号：35081001）；
MEM（HyClone，批号：AC12712264）；
胰酶（GiBco，批号：1931635）
青霉素链霉素（GiBco，批号：2046839）
中性红染液（Sigma，批号：031M1744V）

9.4 试验方法

试验过程无菌操作；

将 L929 细胞培养在含 10%胎牛血清和抗生素（青霉素 100 U/ml，链霉素 100 μg/ml）的 MEM 培养液中，置于 37℃，5% CO_2 培养箱中培养。用 0.5%胰酶（含 EDTA）消化细胞制备成单细胞悬液，细胞悬液离心（200g，3min），然后将细胞重新分散于培养基中，调整细胞密度为 1.0×10^5 个/ml 的细胞悬液；

接种上述细胞悬液到 6 孔细胞培养板中，每孔 2mL，置 37℃培养箱中（5% CO_2，37℃，>90%湿度）培养 24 小时；

待细胞长成单层后，吸出原来的培养液，分别加入 2ml 未稀释的供试品浸提液、阴性对照浸提液和阳性对照浸提液，置于 37℃，5% CO_2 培养箱中培养 48h；

培养 48h 后，弃去浸提液，在每皿中加入 2ml 中性红，孵育 1h，吸弃中性红，加入 2ml PBS，显微镜观察细胞形态变化；

细胞毒性的大小用反应分级来表示，见表 1。

表 1 反应区分级描述

分级	反应	反应区描述
0	无	个别细胞有颗粒，细胞无裂解，生长状态良好
1	轻微	圆形及离散细胞不超过 20%，细胞无颗粒无形态改变，个别细胞裂解，仅轻微的生长抑制
2	轻度	观察到圆形细胞不超过 50%，细胞无颗粒，无广泛细胞裂解，细胞生长抑制不超过 50%
3	中度	观察到圆形及裂解细胞不超过 70%，细胞层未完全破坏，细胞生长抑制超过 50%
4	重度	细胞层接近或完全破坏

9.5 结果

本次试验结果见表 2 和表 3。

表 2 细胞形态学观察

组别	接种细胞前	与样品接触前	与样品接触 48h 后
阴性对照	个别细胞有颗粒，细胞无裂解，生长状态良好	个别细胞有颗粒，细胞无裂解，生长状态良好	个别细胞有颗粒，细胞无裂解，生长状态良好
阳性对照			细胞层接近或完全破坏
试验样品			个别细胞有颗粒，细胞无裂解，生长状态良好

正常：个别细胞有颗粒，细胞无裂解，生长状态良好

表 3 细胞反应

组别	平行 1	平行 2	平行 3	平均	细胞毒性
试验组	0	0	0	0	无
阴性对照	0	0	0	0	无
阳性对照	4	4	4	4	重度

9.6 评价标准

分级大于 2 判为有细胞毒性。

9.7 结　论

在本次试验条件下，样品医疗卫生用非织造布对 L929 细胞无毒性作用。

10.0 记录存储

所有与本次试验有关的原始数据和记录都被保存在指定的 SDWH 档案文件中。

11.0 保密协议

签订检测委托合同即认为双方接受保密协议。

12.0 试验偏离声明

本次试验严格按照方案执行，未发生影响实验数据有效性的偏离。

180015144061

中国认可
国际互认
检测
TESTING
CNAS L2954

Sanitation & Environment Technology Institute, Soochow University, Final Report

Report Number: SDWH-M201801609-1

In Vitro Cytotoxicity Test of
Non-woven Medical And Hygiene Fabric
using ISO 10993-5: 2009 Test Methods
Test on extracts
MEM with 10% FBS Extract

Sponsor

Jinxinda Textile Technology (Changzhou) Co.,Ltd.

Manufacturer

Jinxinda Textile Technology (Changzhou) Co.,Ltd.

Sanitation & Environment Technology Institute, Soochow University
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Direct: +86 512 65880038, Free: 4001078828, Email: med@sudatest.com

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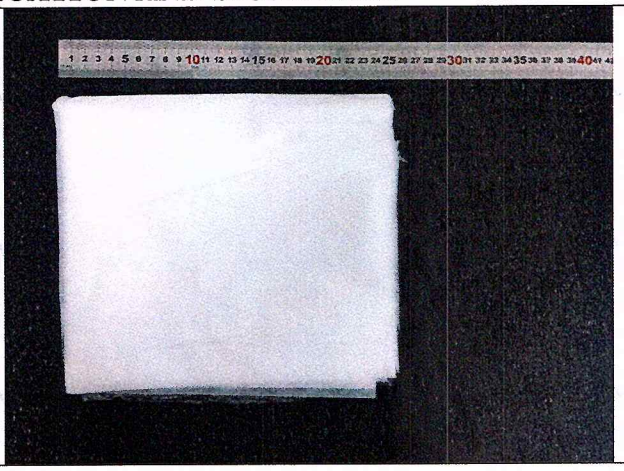
SUPPLEMENTARY EXPLANATION

1. Please apply for rechecking within 15 days of receiving the report if there is any objection.
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 result relate only to the articles tested.
5. The report shall not be reproduced except in full without the written approval of the institute.

STUDY VERIFICATION AND SIGNATURE

Test Article	
Test Article Receipt	2018-05-30
Protocol No	SDWH-PROTOCOL-GLP-M201801609-1
Protocol Effective Date	2018-06-11
Technical Initiation Date	2018-06-12
Technical Completion Date	2018-06-22
Final Report Completion Date	2018-07-02

Edited by : Wang Dehong 2018.07.02
Date

Checked by : Zhu Yuting 2018.07.02
Study Director
Date

Approved by : Wang Lifei 2018-07-02
Authorized signatory
Date

Sanitation & Environment Technology Institute, Soochow University

QUALITY ASSURANCE STATEMENT

This study was conducted in compliance with U.S. Food and Drug Administration regulations set forth in 21 CFR, Part 58.

The sections of the regulations not performed by or under the direction of SDWH, exempt from this Good Laboratory Practice Statement, included characterization and stability of the test article and its mixture with carriers, 21 CFR, Part 58.105 and 58.113.

The Quality Assurance Unit conducted inspections on the following dates. The findings were reported to the Study Director and to SDWH's Management.

INSPECTIONS	DATE OF INSPECTION	DATE REPORTED STUDY DIRECTOR	DATE REPORTED MANAGEMENT
EXPERIMENTAL PROCEDURE	2018-06-19	2018-06-19	2018-07-02
RAW DATA	2018-07-02	2018-07-02	2018-07-02
FINAL REPORT	2018-07-02	2018-07-02	2018-07-02

Quality Assurance Unit : Zhang Yan　　2018-07-02

QA　　Date

1.0 Study Summary

The test article extract was added to L-929 cells and then incubated at 37℃ in 5% CO_2 for 48h to determine the potential cytotoxicity. The results showed the reactivity grades of test article was 0 and the results of control groups showed the test was valid.
Under the conditions of this study, the test article Non-woven Medical And Hygiene Fabric did not show toxicity to L-929 cells.

2.0 Purpose

The purpose of this study was to determine the potential cytotoxicity of the testing article to L-929 cell.

3.0 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)

4.0 Compliance

Good Laboratory Practice Regulations, 21 CFR, Part 58
ISO/IEC 17025:2005 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 No.CNAS L2954
Accreditation Criteria for the competence of Inspection Body (Certification and Accreditation Administration of the People's Republic of China CMA 180015144061)

5.0 Identification of test and control articles

5.1 Test article name: Non-woven Medical And Hygiene Fabric
Test article initial state: Not Sterilized
CAS Code: Not supplied by sponsor (N/S)
Model: N/S
Size: double S hydrophilic non-woven fabric
Lot/ Batch: JXD2018005
Test Article Material: Non Woven Fabric
Packaging Material: N/S
Physical State: Solid
Color: white
Density: N/S
Stability: N/S
Solubility: N/S
Storage Condition: Room Temperature
The information about the test article was supplied by the sponsor wherever applicable;
The Sponsor was responsible for all test article characterization data as specified in the GLP Regulations.
5.2 Negative Control Article Name: Polyethylene, High Density

Manufacturer: U.S. Pharmacopeial Convention (USP)
Size: 3 Strips
Lot/ Batch#: K0M357
Physical State: Solid
Color: White
Stability: Stable at room temperature
Storage Conditions: Room temperature

5.3 Positive Control Article Name: Zinc diethyldithiocarbamate
Manufacturer: Sigma
Size: 25g
Lot/ Batch#: MKBD516V
Concentration: 1%
Solvent: MEM medium, with addition 10% FBS
Date prepared:2018-06-19
Physical State: Solid
Color: White
Storage Condition: 4 ± 2 ℃

6.0 Identification of test system

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

7.0 Justification of the test system

Historically, mouse fibroblast L929 cell has been used for cytotoxicity studies because of its cytotoxic response sensitivity.

8.0 Route of administration

In vitro mammalian cell culture study has been used to historically evaluate the cytotoxicity of biomaterial of medical device.

9.0 Experiment design

9.1 Sample and Control Preparation

Aseptic extracting the test article (test article to volume of vehicle) by MEM medium(10%FBS) according to the table below.Sealed and incubated at 37℃ for 24h.There is no change in the extraction solvent (pre- and post-extraction).Extracts were used immediately after extraction without the process of pH value adjustment, filtering, centrifugation, dilution, etc.

Aseptic Sampling		Sterilization method	Aseptic Extraction In Inert Container			Final Extract	
Sampling Manner	Actually sampling	Cobalt- 60 25kGy	Ratio	Extracts	Condition	pH	Clear or Not
Random sampling	120cm^2		6cm^2 : 1ml	20.0ml	37℃，24h	7.4	Clear

The negative and positive controls were similarly prepared.

9.2 Equipment

Autoclaves (SDWH2204),Calibration Expire(2019-05-15),
Constant temperature shaking table (SDWH2109),Calibration Expire(2018-11-12),
CO_2 Incubator (SDWH021),Calibration Expire(2019-05-15),
Inverted microscope (SDWH037),Calibration Expire(2018-08-28),
Clean bench (SDWH454),Calibration Expire(2019-05-20),
Electronic Balance (SDWH056),Calibration Expire(2019-01-21)
Refrigerator (SDWH-448)

9.3 Reagents

FBS (CORNING , Lot No: 35081001)
MEM (HyClone , Lot No: AC12712264)
Trypsin (GiBco , Lot No: 1931635)
Penicillin, Streptomycin sulfate (GiBco, Lot No: 2046839)
NRU（Sigma,Lot No: 031M1744V）

9.4 Test Method

Aseptic procedures were used handling of cell cultures.
L929 cells were cultured in MEM medium (10% FBS, Penicillin 100 U/ml, Streptomycin sulfate 100 μg/ml) at 37℃ in a humidified atmosphere of 5% CO_2 and then digested by 0.5% trypsin containing EDTA to obtain a 1.0×10^5 cells/ml suspension.
The suspended cells were dispensed at 2ml per well in 6-well plate. Cell morphology was evaluated to verify that the monolayer was satisfactory.
Following incubation, aspirate the culture medium from the monolayers, and replace it with extracts of the Sample Preparation, Negative Control Preparation, or Positive Control Preparation. The serum-supplemented cell culture media extracts are tested in duplicate without dilution (100%). Incubate all cultures for 48 hours at 37 ± 1° in a humidified incubator preferably containing 5 ± 1% of carbon dioxide.
After 48h incubation, aspirate the culture medium and add 2 mL of Neutral Red solution to each plate and incubate for 1h.Pour off the Neutral Red solution and add 2 mL PBS, and examine each culture microscopically.
A useful way to grade test article is given in Table 1.

Table 1 Reactivity grades for Elution Test

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

9.5 Result

The test result is in Table 2 and Table 3.

Table 2 Observation of the Cell morphology

Group	Before inoculation	Before treated with article	48h after treatment
Negative control	Discrete intracytoplasmic granules; no cell lysis	Discrete intracytoplasmic granules; no cell lysis	Discrete intracytoplasmic granules, no cell lysis, no reduction of cell growth.
Positive control			Nearly complete or complete destruction of the cell layers.
Test article			Discrete intracytoplasmic granules, no cell lysis, no reduction of cell growth.

Normal: Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth.

Table 3 Cell reactivity

Group	Parallel 1	Parallel 2	Parallel3	Average	Interpretation
Test Group	0	0	0	0	None cytotoxic
Negative Control	0	0	0	0	None cytotoxic
Positive Control	4	4	4	4	Severely cytotoxic

9.6 Evaluation Criteria

The achievement of a numerical grade greater than 2, based on Tables 1, was considered cytotoxic.

9.7 Conclusion

Under the conditions of this study, the test article Non-woven Medical And Hygiene Fabric did not show toxicity to L-929 cells.

10.0 Record Storage

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

11.0 Confidentiality Agreement

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

12.0 Deviation statement

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