# Test Report

**Date : 2020-05-18** 
**No. : DY20040102**

## TEST FACILITY

STC (Dongguan)

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## SPONSOR

TÜV SÜD Certification and Testing (China) Co., Ltd. Guangzhou Branch
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## SUPPLIER

Dongguan LingJin Precision manufacturing Co. LTD.

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**CONFIDENTIAL**

## STUDY TITLE

Cytotoxicity Test Elution Disposable Medical Face Mask using ISO 10993-5:2009 Test Methods Test on Extract, Minimal Essential Medium with 10% Fetal Bovine Serum Extract

## TEST ARTICLE NAME

Disposable Medical Face Mask

## TEST ARTICLE IDENTIFICATION

CP-MD-2073

CSD NO.: CL2020040038

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# Test Report



**Summary**

The test article, Disposable Medical Face Mask, was evaluated for potential cytotoxic effects. This study was conducted following the guidelines of ISO 10993-5, Biological evaluation of medical devices - Part 5: Tests for in vitro cytotoxicity (2009). A single preparation of the test article was extracted in single strength Minimum Essential Medium at 37 ℃ for 24 hours. The negative control, reagent control, and positive control extracts were similarly extracted. Triplicate monolayers of L-929 mouse fibroblast cells were dosed with each extract and incubated at 37℃ in the presence of 5% $CO_2$ for 24 hours. Following incubation, the monolayers were examined microscopically for abnormal cell morphology and cellular degeneration.

The MEM test extract showed not more than 20% of the cells are round, loosely attached and without intracytoplasmic granules, or show changes in morphology; occasional lysed cells are present; only slight growth inhibition observable. The test article extract met the requirements of the test since the grade was not greater than 1(Slight).

Authorized Signatory Approval: _____

Jonathan Tang

## 1. Introduction

### 1.1 Purpose

The purpose of this study was to determine the potential of a test article to cause cytotoxicity.

### 1.2 Testing Guidelines

This study was based on the requirements of the International Organization for Standardization ISO 10993-5, Biological evaluation of medical device – Part 5: Tests for in vitro cytotoxicity (2009).

### 1.3 Dates

| | |
|---|---|
| Test Article Received: | 2020.04.01 |
| Cells Dosed: | 2020.05.06 |
| Observations Concluded: | 2020.05.08 |

## 2. Identification of Test and Control Articles

The test article provided by the sponsor was identified and handled as described below:

**Table 1: Test Article**

| | |
|---|---|
| Name: | Disposable Medical Face Mask |
| Size: | N.A. |
| CAS: | N.A. |
| Model: | Three-layer ear band mask |
| Lot: | 2020031601 |
| Initial State: | Not Sterilized |
| Strength, Purity and Composition: | PP Spunbond Nonwoven Fabric Containing Copper Oxide, PP Melt-blown Nonwoven Fabric |
| Color: | Orange |
| Physical Description of the Test Article: | Solid |
| Manufacture date: | N.A. |
| Expiration Date: | N.A. |

**Table 2: Negative Control Article**

| | |
|---|---|
| Name: | High Density Polyethylene |
| Lot: | C-161 |
| Source: | Hatano Research Institute, Food and Drug Safety Center |
| Component: | High Density Polyethylene Film |

**Table 3: Positive Control Article**

| | |
|---|---|
| Name: | ZDBC |
| Lot: | B-172K |
| Source:<br>Component: | Hatano Research Institute, Food and Drug Safety Center<br>0.25% ZDBC Polyurethane Film |

**Table 4: Ancillary Materials**

| | |
|---|---|
| Growth Media: | Single strength Minimum Essential Medium supplemented with 10% fetal bovine serum, 1% antibiotics (100 U/mL penicillin, 100 μg/mL streptomycin) |
| Formulation: | 44.5 mL MEM+ 5 mL FBS+0.5 mL antibiotics |

**Table 5: Extraction Vehicle**

| | |
|---|---|
| Name: | MEM |

**Table 6：Ragents**

| Name | Brand | Lot |
|---|---|---|
| MEM | HyClone | AE29146282 |
| FBS | GiBco | 42F1294K |
| Penicillin, Streptomycin | GiBco | 2076673 |

## 3. Test System

### 3.1 Test System and Justification of Test System

Mammalian cell culture monolayer consisting of L-929 mouse fibroblast cells (ATCC Number: CCL-1, Lot Number: 70001022) was used. In vitro mammalian cell culture studies have beenused historically to evaluate cytotoxicity of biomaterials and medical devices.

### 3.2 Test System Management

L-929 mouse fibroblast cells were propagated and maintained in flasks containing IX MEM at 37℃ with 5% carbon dioxide ($CO_2$). For this study, a 6-well plate was seeded with $4.5 \times 10^5$ cells/well and incubated at 37℃ (humidified) with 5% $CO_2$ to obtain semi-confluent monolayers of cells prior to use. Aseptic procedures were used in the handling of the cell cultures following approved STC Standard Operating Procedures.

## 4. Method

### 4.1 Test and Control Article Preparation

The test articles were measured and calculated. The preparations of the test article and the negative control were subjected to the extraction conditions as described below. The extracts were continuously agitated during extraction. The MEM extraction method was conducted in the presence of serum to optimize extraction of both polar and non-polar components.

**Table 7: Extraction**

| Article | Extraction Ratio | Article Amount | Volume of Vehicle | Extraction Condition |
|---|---|---|---|---|
| Test Article | 3 $cm^2$:1 mL | 332.5 $cm^2$ | 110.8mL | 37±1℃ for 24±2 h |
| Negative Control | 3 $cm^2$:1 mL | 18 $cm^2$ | 6mL | 37±1℃ for 24±2 h |
| Positive Control (ZDEC) | 6 $cm^2$:1 mL | 36 $cm^2$ | 6mL | 37±1℃ for 24±2 h |
| Reagent Control | Not Applicable | Not Applicable | 10 mL | 37±1℃ for 24±2 h |

The following table contains a description of the test and control article extracts before and after extraction.

**Table 8: Condition of Extracts**

| Vehicle | Time Observed | Extract | Condition of Extracts | | |
|---|---|---|---|---|---|
| | | | Color | Clarity | Particulates |
| MEM | Before Extraction | Test Article | Pink | Clear | None |
| | | Negative Control | Pink | Clear | None |
| | | Positive Control (ZDEC) | Pink | Clear | None |
| | | Reagent Control | Pink | Clear | None |
| | After Extraction | Test Article | Pink | Clear | None |
| | | Negative Control | Pink | Clear | None |
| | | Positive Control (ZDEC) | Pink | Clear | None |
| | | Reagent Control | Pink | Clear | None |
| | Prior to Use | Test Article | Pink | Clear | None |
| | | Negative Control | Pink | Clear | None |
| | | Positive Control (ZDEC) | Pink | Clear | None |
| | | Reagent Control | Pink | Clear | None |

There appeared to be no visible changes to the test article during the extraction process. The extracts were tested immediately following extraction. The extracts were not centrifuged, filtered, or otherwise altered prior to dosing.

### 4.2 Test Procedure

Triplicate culture wells were selected which contained a subconfluent cell monolayer. The growth medium contained in the triplicate cultures was replaced with 1.5mL of the test extract in each well. Similarly, the growth medium in triplicate 6-wells plate was replaced with 1.5 mL of the reagent control, the negative control and the positive control extracts. The wells of each plate were labeled with the appropriate lab number or control and the replicate number. Each plate was labeled with the test code and the dosing date. The wells were incubated at 37 ℃ in 5%$CO_2$ for 24hours.

Following incubation, the cells were examined microscopically to evaluate cellular characteristics and percent lysis.

## 5. Evaluation and Statistical Analysis

Scoring for cytotoxicity will be based on the following criteria:

**Table 9: Test Scoring**

| Grade | Reactivity | Conditions of all Cultures |
|---|---|---|
| 0 | None | Discrete intracytoplasmic granules, no cell lysis, no reduction of cell growth. |
| 1 | Slight | Not more than 20% of the cells are round, loosely attached and without intracytoplasmic 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 intracytoplasmic 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 completely destroyed, but more than 50% growth inhibition observed. |
| 4 | Severe | Nearly complete or complete destruction of the cell layers. |

For the test to be valid the reagent control and the negative control extracts must have had a reactivity of none (grade 0) and the positive control extract must have been a grade 3 or 4. Percent rounding and percent cells without intracytoplasmic granules are not evaluated in the event of 100% lysis. The test article extract met the requirements of the test if the biological response was less than or equal to grade 2 (mild). The test would have been repeated if the controls did not perform as anticipated.

All times and temperatures reported herein are approximate and are within ranges established by the external standards described in the References section of this report and/or STC standard operating procedures.

## 6. Results

All system suitability criteria were met, indicating a valid test assay.

**Table 10 - Individual Test Data**

| Well | Conditions of all Cultures | Grade | Reactivity |
|---|---|---|---|
| Test (1) | Not more than 20% of the cells are round, loosely attached and without intracytoplasmic granules, or show changes in morphology; occasional lysed cells are present; only slight growth inhibition observable. | 1 | Slight |
| Test (2) | Not more than 20% of the cells are round, loosely attached and without intracytoplasmic granules, or show changes in morphology; occasional lysed cells are present; only slight growth inhibition observable. | 1 | Slight |
| Test (3) | Not more than 20% of the cells are round, loosely attached and without intracytoplasmic granules, or show changes in morphology; occasional lysed cells are present; only slight growth inhibition observable. | 1 | Slight |
| NegativeControl (1) | Discrete intracytoplasmic granules, no cell lysis, no reduction of cell growth. | 0 | None |
| NegativeControl (2) | Discrete intracytoplasmic granules, no cell lysis, no reduction of cell growth. | 0 | None |
| NegativeControl (3) | Discrete intracytoplasmic granules, no cell lysis, no reduction of cell growth. | 0 | None |
| PositiveControl (1) | Nearly complete or complete destruction of the cell layers. | 4 | Severe |
| PositiveControl (2) | Nearly complete or complete destruction of the cell layers. | 4 | Severe |
| PositiveControl (3) | Nearly complete or complete | 4 | Severe |

| | | | |
|---|---|---|---|
| | destruction of the cell layers. | | |

Note: 1, 2, and 3 indicate duplication

## 7. Conclusion

The MEM test extract showed not more than 20% of the cells are round, loosely attached and without intracytoplasmic granules, or show changes in morphology; occasional lysed cells are present; only slight growth inhibition observable. The test article extract met the requirements of the test since the grade was not greater than 1(Slight).

Results and conclusions apply only to the test article tested. Any extrapolation of these data to other articles is the sponsor's responsibility.

## 8. Records

All raw data pertaining to this study and a copy of the final report are retained in designated STC archive files in accordance with STC SOPs.

## 9. ISO Compliance

All procedures were compliance to ISO 17025.

## 10. References

International Organization for Standardization (ISO) 10993-1, Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process (2018).

International Organization for Standardization (ISO) 10993-5, Biological evaluation of medical devices - Part 5: Tests for in vitro cytotoxicity (2009).

International Organization for Standardization (ISO) 10993-12, Biological evaluation of medical devices - Part 12: Sample preparation and reference materials (2012).

International Organization for Standardization (ISO) 17025, General requirements for the competence of testing and calibration laboratories (2017).

**Appendix 1 – Photograph(s) of Test Articles**


******* END OF TEST REPORT *******

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