「“NoGerm” General Purpose Disinfectant (“諾必淨”一般醫療器材用消毒劑)」 Cytotoxicity Test

Final Report

Study Number: TW025-23072L01

Sponsor Name: Nanoplus Life Biomedical Technology Co., Ltd.

Address: 9 F., No. 179, Sec. 2, Tiding Blvd., Neihu Dist., Taipei City 114735, Taiwan (R.O.C.)

Test Facility: Chang Bing Show Chwan Memorial Hospital Animal Center (CBSCMHAC)

Address: No. 6, Lugong Rd., Lukang Township, Changhua County 505, Taiwan (R.O.C.)

Test Facility Approval

Quality Assurance Auditor

Chia-Yun Tsai

Signer Name: Chia-Yun Tsai, MS

May 10, 2024

Date

Study Director

Pei-Jing Chen

Signer Name: Pei-Jing Chen, MS

May 10, 2024

Date

Facility Manager

Chi-Jung Yeh

Signer Name: Chi-Jung Yeh, MT

May. 10. 2024

Date

Good Laboratory Compliance Statement

This study was conducted in compliance with the Organisation for Economic Co-operation and Development (OECD)-Principles of Good Laboratory Practice(GLP) and Taiwan Food and Drug Administration -Good Laboratory Practice for Nonclinical Laboratory Studies.

The laboratory is exempt from the following provisions: Sections 6.2.2, 6.2.3, 6.2.4, 6.2.5 in Taiwan Food and Drug Administration -Good Laboratory Practice for Nonclinical Laboratory Studies and The Organization for Economic Co-operation and Development (OECD)-Principles of Good Laboratory Practice(GLP). Characterization and verification of the test item identity, strength, purity, stability, composition, method of synthesis, fabrication, derivation or other characteristics provided from sponsor, which are the responsibility of the sponsor.

Study Director

Pei-Jing Chen

Signer Name: Pei-Jing Chen, MS

May 10, 2024.

Date

Quality Assurance Statement

Study Number TW025-23072L01

Study Name "NoGerm" General Purpose Disinfectant ("諾必淨"一般醫療器材用消毒劑)

This study has been divided into several phases for inspection. Using a random sampling approach, each of these phases was monitored by quality assurance representative over a series of inspections. Related procedures, equipment records, and documentation are examined in order to assure that the study is performed in accordance with GLP guidelines from the regulatory body, such as the Taiwan Food and Drug Administration and OECD.

The following list describes the inspection date, inspection type, inspection phase, and report dates of inspections conducted by Quality Assurance in this study.

INSPECTION DATE	INSPECTION TYPE	STUDY PHASE	DATE REPORTED TO STUDY DIRECTOR	DATE REPORTED TO FACILITY MANAGER
Mar. 27, 2024	Study-Based inspections	Draft Study Plan Review & Operator's Qualification	Mar. 27, 2024	Mar. 27, 2024
Apr. 19, 2024	Study-Based inspections	Quantitative Evaluation	Apr. 19, 2024	Apr. 19, 2024
May 06, 2024	Study-Based inspections	Raw Data Review	May 06, 2024	May 06, 2024
May 09, 2024	Study-Based inspections	Final Report Review	May 09, 2024	May 09, 2024

I accept the responsibility for conducting inspection to assure that the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Quality Assurance Auditor

Chia-Yun Tsai

Signer Name: Chia-Yun Tsai, MS

May 10, 2024

Date

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Appendix

Summary

The present study was to evaluate the in vitro cytotoxicity of extract from the test item “NoGerm” General Purpose Disinfectant(“諾必淨”一般醫療器材用消毒劑) after 24h treatment. This study was conducted based on the requirements of ISO 10993 (2009), "Biological evaluation of medical devices - Part 5: Tests for in vitro cytotoxicity" to establish a cytotoxicity test. The cell morphology of mouse fibroblast cell line (L929) and the confluency of monolayer were scored under the microscope for qualitative determination. The MTT assay was performed in order to check the cytotoxicity of the test item extract quantitatively. Both qualitative and quantitative results showed that no cytotoxicity of the test item extract was observed in this study.

1. Objectives

Due to potential varying degrees of test item with the human body during use, careful testing and assessment of the safety of test item in manufacturing processes must be conducted to avoid harm from potentially hazardous substances. ISO 10993 (2009) "Biological evaluation of medical devices - Part 5: Tests for in vitro cytotoxicity" to assess whether the test item induces cytotoxicity and to evaluate whether components of medical devices or materials may affect cellular metabolic activity, providing a method for evaluating the safety of potential cytotoxicity of medical devices and contact materials for human use.

2. Test Facility

2.1.Name:

Chang Bing Show Chwan Memorial Hospital Animal Center (CBSCMHAC)

2.2.Address:

1F., No. 6, Lugong Rd., Lukang Township, Changhua County 505, Taiwan (R.O.C.)

3. Personnel

Associates involved in this study was appropriately qualified and trained.

3.1.Study Director:

Pei-Jing Chen, MS.

Phone: +886-4-7813888#73630

E-mail: peijing69458707@gmail.com

Address: 3F., No. 6, Lugong Rd., Lukang Township, Changhua County 505, Taiwan (R.O.C.)

3.2.Study Personnel:

De-Wei Lai, Ph.D.

Pei-Jing Chen, MS

3.3.Study Location:

Cell culture room	Preparation of extracts and cytotoxicity test

4. Sponsor Information

4.1.Name:

Nanoplus Life Biomedical Technology Co., Ltd

4.2.Address:

9 F., No. 179, Sec. 2, Tiding Blvd., Neihu Dist., Taipei City 114735, Taiwan (R.O.C.)

4.3.Sponsor's Representative and contact information:

Tzu-Ying Hung (洪慈營)

Phone: (02)2656-0555

Email: Cecilia@nanoplustech.com

5. Study Schedule

1. Estimated Study initiation date:	Apr. 02, 2024
2. Test item receipt date:	Feb. 16, 2024
3. Experimental starting date:	Apr. 10, 2024
4. Experimental completion date:	Apr. 19, 2024
5. Study completion date:	Actual dates will be presented in the Final Report

6. Test Item

6.1.Test Item:

Name of Test Item: “NoGerm” General Purpose Disinfectant (“諾必淨”一般醫療器材用消毒劑)

Receipt Date: Feb. 16, 2024

Lot Number: 51224010004

Test Item No.: TW025-23072L01 -51224010004 (1) (test)

TW025-23072L01 -51224010004 (2) (retention)

Type: Medical Devices，Category：Class 1; Surface Device

External Features: Liquid

Surface Area: Not Available

Thickness: Not Available

Concentration: N/A

Purity: N/A

Expiry Date: Jan. 30, 2027

Manufacture Date: Jan. 31, 2024

Amount: Test: 1bot* 4L, Retention: 1 bot* 4L

Major Components: Electrolyzed water

Sterilization: No

Color: Colorless

Stability: N/A

pH value: N/A

Storage Condition: Room Temperature (SC-01 region)

Cut or Not: N/A

Extraction: By Weight: (0.2±10%)g/mL; Condition: (50±2)℃ (72±2)Hours

Test Conditions: No Dilution

Item Return: No, The remaining materials considered as retention? No

(Processing Method: Remaining materials is processed by CBSCMHAC)

Attachment: No Attachment

Other: Relevant information is shown in Appendix 1 and Appendix 2

7. Test System

7.1.Test System:

7.1.1. Cell line: L929 mouse fibroblasts，NCTC clone 929 (ATCC_CCL-1，BCRC_RM60091)

7.1.2. Source: Food Industry Research and Development Institute, Bioresource Collection and Research Center (BCRC) (No. 331, Food Road, Hsinchu City, Taiwan (R.O.C.)).

7.1.3. Type: subconfluent monolayer

7.1.4. Growth Property: adherent-cell

7.1.5. Culture Region: Chang Bing Show Chwan Memorial Hospital Experimental Animal Center Cell Culture Room. (3F., No. 6, Lugong Rd., Lukang Township, Changhua County 505, Taiwan (R.O.C.))

7.1.6. Growth Conditions:

i Environment: 37±1℃ in 5±1% CO_2 atmosphere

ii Medium: Eagle minimum essential medium (MEM) supplemented with 10% FBS and 1% Penicillin / Streptomycin solution。

7.2.Justification of Test System: L929 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 (2009).

8. Study Design

This test procedure refers to ISO10993-5 (2009), SOP GLP-TM-20-03 cytotoxicity test-MTT and SOP GLP-TM-20-02 Cytotoxicity test-neutral red uptake (NRU).

8.1.**Reagent:**

8.1.1. Eagle minimum essential medium (MEM), catalog: 11095080, lot: 2645336, supplier: Gibco, expiry date: Jun. 30, 2024
8.1.2. Fetal bovine serum (FBS), catalog: SH30396, lot: AH30687843, supplier: Hyclone, expiry date: Aug. 31, 2027。
8.1.3. 100x Penicillin-Streptomycin (100x P/S), catalog: 15140122, lot: 2585637, supplier: Gibco, expiry date: Jul. 31, 2024
8.1.4. 0.25% trypsin-EDTA, catalog: 25200056, lot: 2661762, supplier: Gibco, expiry date: Apr. 30,2025
8.1.5. 0.4%Trypan blue, catalog: T8154, lot: RNBM0108, supplier: Sigma-Aldrich, expiry date: Dec. 12, 2024
8.1.6. 1x PBS, catalog: 10010023, lot: 2661454, supplier: Gibco, expiry date: May 30, 2025
8.1.7. DPBS, catalog: 14040133, lot: 2813961, supplier: Gibco, expiry date: Oct. 30, 2026
8.1.8. Neutral red, catalog: N2889, lot: RNBL5427, supplier: Sigma-Aldrich, expiry date: Nov. 31, 2024
8.1.9. Dimethyl sulfoxide (DMSO), catalog: D2650, lot: RNBK7774, supplier: Sigma-Aldrich, expiry date: Apr. 30, 2024
8.1.10. MTT cell proliferation/viability assay kit, catalog: 4890-25-K, lot: P356122, supplier: R&D systems, expiry date: Jan. 22, 2025

8.2.**Materials:**

8.2.1. 1000μL Tip, catalog: TF112-1000-Q, lot: 1364140, supplier: QSP, expiry date: Jan. 08, 2028
8.2.2. 200μL Tip, catalog: TF140-200-Q, lot: 20230662, supplier: QSP, expiry date: Jun. 17, 2025
8.2.3. 10μL Tip, catalog: TLR102RS-Q, lot: 22390214, supplier: QSP, expiry date: Sep. 25, 2027
8.2.4. 25mL serological pipette, catalog: 1044001, lot: 28422140, supplier: Saining, expiry date: Oct. 10, 2025
8.2.5. 10mL serological pipette, catalog: 91010, lot: CB3G17A91010, supplier: SPL, expiry date: Jul. 31, 2028
8.2.6. 5mL serological pipette, catalog: 91005, lot: CB2A03A91005, supplier: SPL, expiry date: Jan. 30, 2027
8.2.7. 75T flask, catalog: 70075, lot: FB3K06A70075, supplier: SPL, expiry date: Nov. 31, 2028
8.2.8. 50mL centrifuge tube, catalog: 5100050C, lot: 50C2G1128B, supplier: CAPP, expiry date: Aug. 31, 2024
8.2.9. 15mL centrifuge tube, catalog: CT-15PL-TW, lot: 06523601, supplier: Protech, expiry date: Mar. 27,

2026

8.2.10. Whirl-pak, catalog: B01065, lot: 124459, supplier: Nasco, expiry date: Apr. 25, 2027
8.2.11. 24 well plate, catalog: 92024, lot: 20190023, supplier: TPP, expiry date: Jan. 31, 2025
8.2.12. 96 well plate, catalog: 3860-096, lot: 0112201, supplier: IWAKI, expiry date: Jan. 10, 2027
8.2.13. HDPE closure, catalog: 339650, lot: N7GF7F7116, supplier: Thermo, expiry date: Jul. 06, 2028
8.2.14. 75% EtOH, lot: HA221127, supplier: TOUN-SHIN CO., LTD., expiry date: Nov. 21, 2026

8.3.**Equipment:**

8.3.1. Biological safety cabinet (BSC), model: AC2-3S2, supplier: ESCO, equipment number: BSC-12, calibration date: Feb. 02, 2024
8.3.2. CO_2 incubator, model: CCL-170B-9-UV, supplier: ESCO, equipment number: INB-06, calibration date: Mar. 18, 2024
8.3.3. Incubator, model: K-600DT, supplier: YIHDER, equipment number: SI-02, calibration date: Mar. 18, 2024
8.3.4. Ultracentrifuge, model: 1236R, supplier: Scanspeed, equipment number: ULC-04, calibration date: Mar. 18, 2024
8.3.5. Electronic temperature record, model: KTT-220, supplier: KIMO, equipment number: ETR-02 calibration date: Feb. 27, 2024
8.3.6. ELISA reader, model: M200pro, supplier: TECAN, equipment number: ELIR-01 calibration date: Jul. 11, 2023
8.3.7. Microbalance, model: PA224C, supplier: OHAUS, equipment number: BAL-05, calibration date: Mar. 19, 2024
8.3.8. 1000µl pipette, supplier: Sorenson, equipment number: P1mL-11, calibration date: Jul. 11, 2023
8.3.9. 200µl pipette, supplier: Sorenson, equipment number: P200-11, calibration date: Jul. 11, 2023
8.3.10. 100µl pipette, supplier: Sorenson, equipment number: P100-11, calibration date: Jul. 11, 2023
8.3.11. 20µl pipette, supplier: Sorenson, equipment number: P20-11, calibration date: Jul. 11, 2023
8.3.12. Water bath, model: BH-230D, supplier: YIHDER, equipment number: WB-04, calibration date: N.A
8.3.13. Pipette Aid, model: DM4-040-101-NC, supplier: Thermo, equipment number: PIPA-07, calibration date: N.A
8.3.14. Inverted microscope, model: Primo vert, supplier: Zeiss, equipment number: PCM-05, calibration date: N.A

8.4.**Test Procedure:**

8.4.1. In accordance with SOP GLP-EQ-10-01 Biological Safety Cabinet, relevant sampling procedures were performed within the BSC, and measures to prevent cross-contamination were strictly adhered to.

8.4.2. Opened the BSC and followed the operating procedures outlined in SOP GLP-EQ-10-01 Biological

safety cabinet.

8.4.3. Once the startup was complete, transferred the required materials and reagents sprayed with 75% EtOH into the BSC.

8.5.**Preparation of complete medium:**

8.5.1. The materials and relevant operational steps for this experiment were recorded in GLP-RM-20-02-01F Reagent Preparation.

8.5.2. 44.5mL of MEM, 5mL of FBS, and 0.5mL of 100x P/S were added to a 50 mL centrifuge tube. The tube was gently shaken to mix thoroughly, preparing a total volume of 150 mL of complete medium.

8.5.3. The centrifuge tube was stored at 4±2°C. It should be used within two weeks.

8.6.**Preparation of test item and control item:**

8.6.1. **Test item extraction:**

8.6.1.1. The extraction steps referred to SOP GLP-TM-10-03 Test Item Extraction and ISO10993-12 (2021).

8.6.1.2. Using MEM complete medium as the extraction solution, The extraction were performed in close inert containers according to the extraction ration of (0.2±10%)g: 1 mL.

8.6.1.3. The extraction conditions were 50±1°C for 72±2 hrs.

8.6.1.4. After extraction, the extract solution were used immediately; the appearance of test item extract was clear, particulate-free and no color changed.

8.6.1.5. The test item extraction solutions were tested at two concentrations: 100% extraction solution and 50% extraction solution.

8.6.2. **Control item extraction:**

8.6.2.1. Blank: 10mL of MEM complete medium.

8.6.2.2. Positive control: MEM complete medium inclusive of 10% (v/v) DMSO.

8.6.2.3. Negative control: HDPE closure (Based on the ISO 10993-12:2021, the extraction ratio was 0.2 g/mL).

8.6.2.4. The extraction conditions were 50±1°C for 72±2 hrs.

8.7.**Cell culture:**

8.7.1. The materials and relevant operational steps for this experiment were recorded in GLP-TM-00-01-01F Cell Sub-Culturing Operation Recorded.

8.7.2. L929 cells were cultured in MEM complete medium at 37±1℃ in a humidified atmosphere of 5±1% CO_2, Up to 80% cell growth was observed by microscope. Then they were digested by 0.25% trypsin containing EDTA to obtain single-cell suspension. Subculture was performed until obtaining passage number 2 prior to use.

8.8.**Cytotoxicity test**

8.8.1. **Qualitative analysis (neutral red)**

8.8.1.1. The materials and relevant operational steps for this experiment were recorded in GLP-TM-20-02-01F Cytotoxicity Test-Neutral Red Uptake (NRU) Operation Record.

8.8.1.2. 5×10^4 of L929 mouse fibroblast cells were seeded in 24-well culture plates and then incubated at 37±1℃ in 5±1% CO_2 atmosphere for 24±2 hrs.

8.8.1.3. The following day, the confluency and morphology of the cells were observed and found to be approximately 80% confluent and normal. The original culture medium was removed and replenished 0.5mL of blank, positive control (PC), negative control (NC), 100% extraction solution (100% test) and 50% extraction solution (50% test) in each of culture well.

	1	2	3	4	5	6
A	Blank	PC	NC	100% test	50% test	
B	Blank	PC	NC	100% test	50% test	
C	Blank	PC	NC	100% test	50% test	
D						

8.8.1.4. Each treatment was carried out in triplicate. Then, the 24-well culture plates were subsequently incubated at 37±1℃ in 5±1% CO_2 atmosphere for 24±2 hrs.

8.8.1.5. The NR medium was incubated overnight at 37±1°C.

8.8.1.6. After 24±2 hrs of incubation, the cells were examined under inverted microscope for morphological evidence of cytotoxicity.

8.8.1.7. The NR medium should be centrifuged at 600g for 10 min (to remove NR crystals) before adding to the cells.

8.8.1.8. The cells were washed with 0.5mL DPBS. The washing solution was removed by gentle tapping. Then, 0.5ml NR medium was added, and the cells were incubated at 37±1°C in a humidified

atmosphere of 5±1% CO_2 for 3h.

8.8.1.9. The cells of each well were scored by the change of cell morphology and viability under inverted microscope in accordance with criteria using a grading scheme according to ISO 10993-5:2009(E).

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 is observed.
2	Mild	Not more than 50% of the cells are round, devoid of intracytoplasmic granules; not more than 50% growth inhibition is observed.
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 is observed.
4	Severe	Nearly complete or complete destruction of the cell layers.

8.8.2. **Quantitative analysis (MTT assay)**

8.8.2.1. The materials and relevant operational steps for this experiment were recorded in GLP-TM-20-03-01F Cytotoxicity Test-MTT Operation Record.

8.8.2.2. 0.1mL of MEM complete medium was added around the wells of the 96-well plate.

8.8.2.3. L929 mouse fibroblast cells were seeded in 96-well culture plates with the number of 1×10^4 cells, and then incubated at 37±1℃ in 5±1% CO_2 atmosphere for 24±2 hrs.

8.8.2.4. The following day, confluency and morphology of the cells were observed and found to be approximately 80% confluent and normal, the original culture medium was removed and replenished 0.1mL of blank (B), positive control PC), negative control (NC), 100% extraction solution (100% test) and 50% extraction solution (50% test) in each of culture well.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		Blank	PC	NC	100% test	50% test					Blank	
C		Blank	PC	NC	100% test	50% test					Blank	
D		Blank	PC	NC	100% test	50% test					Blank	
E		Blank									Blank	
F		Blank									Blank	
G		Blank									Blank	
H												

8.8.2.5. Each treatment, except for the blank, should have at least three replicates. The blank located in Row 2 and Row 11, should have a total of 12 replicates.

8.8.2.6. Then the test plates were subsequently incubated at 37±1℃ in 5±1% CO_2 atmosphere for 24±2 hrs.

8.8.2.7. After 24±2 hrs of incubation, the cells were examined under inverted microscope for morphological evidence of cytotoxicity.

8.8.2.8. At the end of the incubation period, the B, PC, NC, 100% test, 50% test were removed and replenished 0.1mL of fresh MEM complete medium.

8.8.2.9. Then 10μL MTT reagent was added into each well. The reaction was performed at 37±1°C in 5±1% CO_2 atmosphere for 2-3 hrs.

8.8.2.10. 0.1mL of detergent reagent was added into each well. The cells were incubated in dark for 2-4hrs before measuring the absorbance.

8.8.2.11. The absorbance of test samples was measured at 570 nm (reference wavelength: 630 nm) by a ELISA reader (Infinite M200Pro, TECAN).

8.8.2.12. The raw data was saved in a file format (e.g. ASCII, TXT, XLS) appropriate for further analysis.

9. Results Analysis

9.1. The absorbance of test samples was represented as mean ± standard deviation (SD).

9.2. A test met the acceptance criteria if the mean OD570 of blanks was≥ 0.2.

9.3. The left and the right mean of the untreated blanks did not differ by more than 15% from the mean of all untreated blanks.

9.4. Compared to the blank (as 100% viability), the percentage of viability and mortality was calculated by the following formula:

$$\text{Viability (\%)} = \frac{\text{OD570s}}{\text{OD570b}} \times 100\%$$

Mortality (%) = 100 – Viability (%)

Where

OD570s was the mean value of the measured absorbance of the test samples; OD570b was the mean value of the measured absorbance of the blank (B).

9.5. If the test sample viability was reduced to < 70% of the blank, it was considered to have cytotoxic

potential. The 50% extract of the test sample was required to have at least the same or a higher viability than the 100% extract; otherwise, the test should have been repeated.

9.6. The positive control showed a positive cytotoxic response of >30%.

9.7. The average viability of the negative control group was judged as non-cytotoxic.

10.Results

10.1. Extraction

Group	Extraction Ratio	Weight of Test Item	Extraction Volume	Condition of Extraction (Estimated)	Condition of Extraction (Actual)
Blank	-	-	10.0mL	50±1℃; 72±2 hrs	49.2-50.4℃; 23 hrs 52 mins
positive control	10%	1mL	9.0mL		
negative control	(0.2±10%)g/mL	0.99g	4.96mL		
Test item	(0.2±10%)g/mL	2mL	8.0mL		

10.2. Extraction Condition

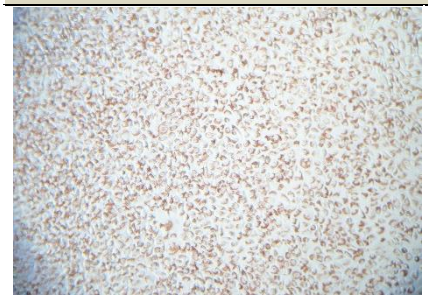
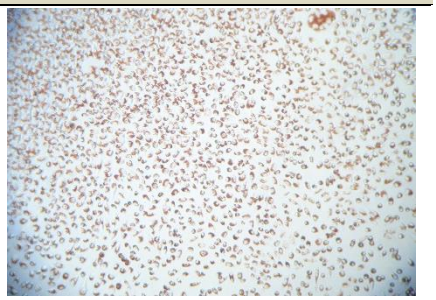
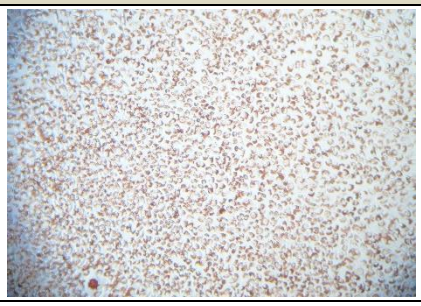
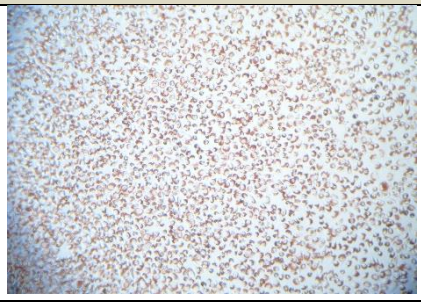
Group	Observation Point	Extraction State		
		Color	Clarity	Precipitate
Blank	Before Extract	red	clear	No
	After Extract	fuchsia	clear	No
positive control	Before Extract	red	clear	No
	After Extract	fuchsia	clear	No
negative control	Before Extract	red	clear	No
	After Extract	fuchsia	clear	No
Test item	Before Extract	fuchsia	clear	No
	After Extract	fuchsia	clear	No

10.3. **CO_2 Incubator Condition**

	State	Temperature (℃)	CO_2 Concentration (%)	Time
Cell culture	-	36.1-37.3	4.72-5.54	-
Neutral Red	Seeding cell	36.8-38.0	5.0-5.34	23hrs 40mins
	treatment	36.5-37.3	5.02-5.12	23hrs 28mins
	stain	36.7-37.1	5.05-5.11	3hrs
MTT	Seeding cell	36.8-38.0	5.0-5.34	23hrs 40mins
	treatment	36.5-37.3	5.02-5.12	23hrs 28mins
	MTT reagent	36.7-37.1	5.05-5.11	2hrs 49mins
	detergent reagent	RT	-	2hrs 56mins

10.4. **Qualitative Analysis (Neutral Red)**

10.4.1. The observed cell morphology of L929 cells after being treated for 24h under 100x inverted microscope.

blank	**positive control**	**negative control**
		
100% test item extraction solution		**50% test item extraction solution**
		

10.4.2. Cytotoxicity evaluated using neutral red stain.

Group	Grade		
blank	0	0	0
positive control	4	4	4
negative control	0	0	0
100% test item extraction solution	0	0	0
50% test item extraction solution	0	0	0

10.4.3. Qualitative determination

The morphology of blank and negative control cells showed long spindle shape with obvious lamellipodia and filopodia instead of lysed, rounded shape, and inhibited growth. However, positive control-treated cells showed a nearly complete rounded lysed morphology; cell layers were almost completely destroyed, and growth inhibition was observed. The result of test sample showed the same long spindle shape as blank and negative control.

Based on the results observed under the microscope, the percentages of rounded or lysed cells of blank (B), positive control (PC), negative control (NC), 100% test item extraction solution (100% test) and 50% test item extraction solution (50% test) were evaluated at 0%, 100%, 0%, 0%, and 0% respectively. Therefore, the cell morphology of B, PC, NC, 100% test and 50% test was graded at 0, 4, 0, 0, and 0.

10.5. **Quantitative analysis (MTT assay)**

10.5.1. Optical density readings at 570 nm

Group		$OD_{570\ nm}$						
		1	2	3	4	5	6	Mean
blank	Row2	0.858	0.881	0.801	0.910	0.838	0.818	0.851
	Row11	0.727	0.831	0.841	0.889	0.896	0.832	0.836
positive control		0.099	0.133	0.138				0.123
negative control		0.802	0.830	0.919				0.850
100% test item extraction solution		0.889	0.925	0.936				0.917
50% test item extraction solution		0.828	0.851	0.881				0.853

10.5.2. The results of MTT assay for evaluation of cell viability

Group	Absorbance ($OD_{570\ nm}$)	Viability (%)	Mortality (%)
blank	0.843± 0.050	100	0
positive control	0.123± 0.021	14.59	85.41
negative control	0.850± 0.061	100.78	0
100% test item extraction solution	0.917± 0.024	108.67	0
50% test item extraction solution	0.853± 0.027	101.15	0

10.5.3. The $OD_{570\ nm}$ of blank

The blank (Row 2/11) with the difference of blank being 7.49% (≤15%). This test method meets the acceptance criteria.

	Absorbance ($OD_{570\ nm}$)	Mean of all blank	Difference of blank (%)
Row2	0.851± 0.040	0.843	0.90
Row11	0.836± 0.061		

10.5.4. Quantitative determination

The cell viability was evaluated after L929 cells treated with blank (B), positive control (PC), negative control (NC), 100% test item extraction solution (100% test) and 50% test item extraction solution (50% test) for 24h through MTT cell proliferation/viability assay.

Based on the results of MTT assay for evaluation of cell viability, the absorbance of B, PC, NC, 100% test and 50% test were 0.843± 0.050, 0.123± 0.021, 0.850± 0.061, 0.917± 0.024 and 0.853± 0.027 respectively; the cell viability represented 100%, 14.59%, 100.78%, 108.67% and 101.15%; the mortality showed 0%, 85.41%, 0%, 0% and 0%.

10.6. Conclusion

The cell morphology of positive control was average graded at 4, the test item extraction solution was average graded at 0. The average cell viability for the positive control is 14.59% and the test item extraction solution is 100%.

According to the results of qualitative and quantitative assays, the results showed “Zero” reactivity. Therefore, none *in vitro* cytotoxicity could be considered in the extract solution of the test item “NoGerm” General Purpose Disinfectant (“諾必淨”一般醫療器材用消毒劑).

11.Deviation Statement

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

12.Study Documents and Archives

The study pertinent records to be maintained were included, but were not limited to, the following objects:

- Signed Study Plan and amendments (as needed)
- Deviations from the Study Plan or SOPs
- Material record (e.g. Test Item/ Control Item/ Specimen Information Form, Receiving Item Confirmation Form, Controlled Item Request Form, Control Area Access Record, Item Return Processing Record and etc.)
- Raw data of experimental results(e.g. Extraction Test Procedure, Reagent Preparation, Cell Cryogenic Tubes Storage and Access Recorded draft, Cell Thawing Operation Recorded draft, Cell Sub-Culturing Operation Recorded draft, Cytotoxicity Test-Neutral Red Uptake (NRU) Operation Record draft, Cytotoxicity Test-MTT Operation Record, Equipment Use Record draft and cell morphology images.)
- Correspondence between the CBSCMHAC and the Sponsor Application Form
- A copy of Final Report and amendments (as needed)

The above-mentioned data and documents will be maintained in the archives at Animal Center of Chang Bing Show Chwan Memorial Hospital for at least 6 years after report finalization. The retention of test item will be maintained in the storage cabinet (SC-01) in the control area of the test item management room at room temperature. The storage period of the test item will be reserved for at least 6 years after the final report becomes effective or be withdrawn after the validity period of the test item.

13.References

12.1.Taiwan Food and Drug Administration -Good Laboratory Practice for Nonclinical Laboratory Studies. (2019)

12.2.The Organisation for Economic Co-operation and Development (OECD)- Principles of Good Laboratory Practice (GLP). (1997)

12.3.ISO 10993-5 Biological evaluation of medical devices —Part 5:Tests for in vitro cytotoxicity

12.4.ICH. Validation of Analytical Procedured: Text and Methodology Q2 (R1). (2005)

12.5.SOP GLP-EQ-20-01Microbalance (PA224C)

12.6.SOP GLP-EQ-10-01 Biological safety cabinet (AC2-4S9-NS)

12.7.SOP GLP-TM-00-01Cell Culture

12.8.SOP GLP-TM-10-03 Test Item Extraction

12.9.SOP GLP-TM-20-03Cytotoxicity Test-MTT

12.10. SOP GLP-TM-20-02Cytotoxicity Test-Neutral Red Uptake (NRU)

Appendix 1

試驗物質/對照物質/檢體資料表(英文)

Test Item/ Control Item/ Specimen Information Form (English)

Information marked with "*" will be written in the final report, please fill in carefully.

Test/Control ItemNo.	TW025-23072L01 It will be labeled by CBSCMHAC receiving personnel.

Sponsor information			
*SponsorCompanyName	Nanoplus Life Biomedical Technology Co., Ltd.		
*SponsorAddress	9 F., No. 179, Sec. 2, Tiding Blvd., Neihu Dist., Taipei City 114735, Taiwan (R.O.C.)		
*Sponsor's Representative	Ming-Jer Shieh(謝明哲)/ Tzu-Ying Hung(洪慈瑩)		
*Contact Person	Tzu-Ying Hung (洪慈瑩)	Telephone Number	(02)2656-0555
		E-mail	Cecilia@nanoplustech.com
*Test Name	Cytotoxicity Test		
*Estimated Transfer Date	02 / 16 /2024(mm/dd/ yyyy)		
Transfer Method	■Personal delivery □ Post □ By sea/shipping □ Others :__		
Transfer Conditions	■ Room Temperature□Refrigerated □Freezing □ Dry ice □ Liquid Nitrogen□ Others：_____		

■**TEST ITEM/** □**CONTROL ITEM**

Information of Test Item/ Control Item	
* Name ofTestItem/ControlItem	"NoGerm" General Purpose Disinfectant ("諾必淨"一般醫療器材用消毒劑)
* Batch/LotNumber	51224010004
Type	■Medical Devices，Category:Class 1 ■SurfaceDevice □ExternalCommunicatingDevice:CirculatingBlood：□Yes/ □No □ImplantDevice □GasPathways □OtherType： □Food; □Cosmetics Products; □IndustrialChemicals; □Pesticide Products; □Drug; □Others：_____
PackingCondition	□In Bulk; □Intact Packing; (■Bottled□Boxed □Tube□Barrel□ Bag□ Others：__)
Amount(Note2)	Test:1 bottles, volume: 4 L; Retention:1bottles, volume: 4 L
Manufacture Date	01 / 31 / 2024(mm/dd/ yyyy)□No information provided.
Expiry Date(Note3)	01 / 30 / 2027(mm/dd/ yyyy)
* MajorComponents	Electrolyzed water
* Purity/Concentration	Purity:N.A.
	Concentration: NA

試驗物質/對照物質/檢體資料表(英文)

Test Item/ Control Item/ Specimen Information Form (English)

*Description (Note4)	ExternalFeatures: □Regular; □Irregular; ■Liquid; □Powder; □Granule; □Flat; □Other: _____
	Color: Colorless
	Stability: N.A.
	PH values:N.A.
	Sterilization: Has been Sterilized ■NO□YES,IfYes, Please Selectthe FollowingMethod □EO Sterilization□Gamma Sterilization□SteamSterilization□Other_ Thickness：____mm/pcs or ■NotAvailable. Surface Area：□ _____ cm^2/ pcs,□Double Side□Single Side□All Side or ■Not Available. *According to FDA guidance, determine the appropriate amount of test material by using surface area to extractant volume ratios. Mass to extractant volume ratios should only be used if surface area cannot be calculated, or if use of mass will result in a larger sample. In addition, the test will be performed directly with the surface area provided by the sponsor.
* Storage Condition	■RoomTemperature□2~8℃□-20±10℃□-65~-85℃□Liquid Nitrogen □Keep in Dark Place□Avoid Moisture□Others：_____
CutorNot(Note5)	□Yes□No ■N.A. (Liquid, Gel, Powder)
*Solvent /Solubility	Solvent:N.A.
	Solubility:N.A.
Test Conditions (Dilution)	■No Dilution
	□Yes,Test final concentration (%):_____;Recommended Solvent:_____
Item Return (Note6)	■No, The remaining materials considered as retention?□Yes■No □N.A. (Processing Method: Remaining material is processed by CBSCMHAC)
	□Yes, only for un-used test item， Delivery Conditions: □Room Temperature□Refrigerated□Frozen□Dry Ice□ Liquid Nitrogen Recipient:_____, Telephone:_____ Address:_____
Attachment (Note4)	□Certificate of Analysis□Material Safety Data Sheet □Stability Test Result □Product Manual □Others：_____■No Attachment
Extraction	Ratio: □No Extraction □By Surface Area: □(3±10%)cm^2/mL □(6±10%)cm^2/mL ■By Weight: □(0.1±10%)g/mL ■(0.2±10%)g/mL □Others:_____ Condition: □(37±1)℃(24±2)Hours ■(50±2)℃(72±2)Hours □Other：
Others	

試驗物質/對照物質/檢體資料表(英文)
Test Item/ Control Item/ Specimen Information Form (English)

Precautions

- Note1. All information in the form including the blanks which can't be provided are disclosure and should taken responsibility by the sponsor.
- Note2. If the test follow GLP, should provide extra amount of the test/control item with the same lot for retention. If sponsor doesn't provide the retention of test item/control item, the retention of a reserved test item/control item from each lot/batch of test item /control item is the responsibility of the Sponsor.(Example: 1mg/1 mL* 1 bot or $1x10^6$ cell/1 mL*2 vail)
- Note3. For retention, if the effective period is less than CBSCMHAC SOP definition, the test item/control item will be retained till the expiry date. If the expiry date is longer than CBSCMHAC SOP definition, the test item/control item will be retained for the period of CBSCMHAC SOP definition only. If the expiry date remained incomplete, it mentions that sponsor will agree that test facility will determine the earliest date, e.g. Exp.Date: 2021(YYYY), sponsor didn't identify the MM/DD, the expiration date should be 01/01/2021 (means Jan. 01, 2021)
- Note4. Sponsor should determinate, document and confirm the identity, strength, purity, stability, composition, method of synthesis, fabrication, derivation or other characteristics of the test item/control item before study. If the sponsor cannot provide the information, determination and documentation of the test item/control item are the responsibility of the Sponsor.
- Note5. The test item/control item which has been destroyed or cutting will be discarded after the end of experiment unless applicant/sponsor have extra requirement. If the sponsor does not agree or have special requirements, should take the initiative to inform test facility and state the application prior to test. If related losses incurred because applicant/sponsor didn't inform, we will not be liable.
- Note6. If sponsor wants to return the sample, the retention, inconsistencies and biological safety related issue of test item/control item are the responsibility of the Sponsor.
- Note7. Note 'N/A' or 'N.A'if not applicable. Do not leave blank.
- Note8. Test item and control item should be filled individually in "INFORMATION OF TEST ITEM/CONTROL ITEM".
- Note9. GLP test：(1) Corrections and additions to a final report should be in the form of amendments. Amendments should clearly specify the reason forthe corrections or additions. Lot number, test item photos and raw data cannot be amended by sponsor's requirement. (2)One protocol only can generate one report except for translation version. If the report with more than two languages version, we only issue English version protocol. We only issue amendment or additional language GLP report within three years and non-GLP report within one year. (3)The GLP compliance statement will state that we follow TFDA GLP and TAF OECD GLP norm unless sponsor's requirement. Chang Bing Show Chwan Memorial Hospital Experimental Animal Center (CBSCMHAC) have acquired Good Laboratory Practice Statement of Compliance from TFDA and TAF.
- Note10. Please write down it carefully and in detail. "INFORMATION OF TEST ITEM/CONTROL ITEM" will be placed in the protocol and report along with a copy of this official document. If the information is not clear, we will exclude them from GLP statement.
- Note11. The results shown in this test report refer only to the test item(s) tested.
- Note12. If the sponsor is different from the applicant mentioned in the application form, the sponsor agrees that the study-related information, test/control item and application form provided or reply to CBSCMHAC shall be agreed by the sponsor. The report submitted by SGS is only to the applicant.
- Note13. For commissioned tests under GLP specifications, each batch of test item/control item shall comply with the regulations of the planned regulatory agency (for example: FDA/OECD GLP). The test consignor should attach the relevant certificate of the test item, if it cannot be provided, it will be excluded from the GLP statement.

  CBSCMHAC makers no GLP compliance claim for the following items:
  - 1. Sections 6.2.2, 6.2.3, 6.2.4, 6.2.5 in Taiwan Food and Drug Administration -Good Laboratory Practice for Nonclinical Laboratory Studies and The Organization for Economic Co-operation and Development (OECD)- Principles of Good Laboratory Practice(GLP). Characterization and verification of the test article identity and properties provided from sponsor, which are the responsibility of the sponsor.
  - 2. Sections 6.2.6 in Taiwan Food and Drug Administration -Good Laboratory Practice for Nonclinical Laboratory Studies and The Organization for Economic Co-operation and Development (OECD)- Principles of Good Laboratory Practice (GLP). A sample for analytical purposes from each batch of test item should be retained for all studies except short-term studies. Sponsor is responsible for the retention of samples for storage conditions other than room temperature and 4°C refrigeration.

Sponsor'sRepresentative Signature/Date: 01/22/2024

Appendix 2


諾必淨
4L


"諾必淨"一般醫療器械用消毒劑
'NoGerm' General Purpose Disinfectant
LOT 51224010004
MFG 20240131
EXP 20270130