NATIONAL INSTITUTE OF PUBLIC HEALTH
Šrobárova 49/48
Praha 10
100 00
Czech Republic

YOUR REFERENCE:
DATE: March 16, 2020
OUR REFERENCE: 3259/2020
**CTZB 187-3259/20-73, EX 200366**

Hana Bendová, M.Sc., Ph.D.
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Date: March 30, 2020

**Nano Medical s.r.o.**
**5. května 1640/45**
**140 00 Praha 4**

**Subject: EXPERT OPINION on the assessment of human skin irritation.**

**SUBJECT OF APPLICATION:**
Regarding your application of March 16, 2020 for evaluation of human skin irritation, we hereby report:

**SUBMITTED SAMPLE:**
**TM 3/20/73: AntiMicrobe Web R**

**Sponsor:**
**Nano Medical s.r.o., 5. května 1640/45, 140 00 Praha 4 – Czech Republic**

**SUBMITTED DOCUMENTATION:**
Not submitted.

**PERFORMED TESTS:**
SOP 2/3 – Tests for irritation and delayed-type hypersensitivity (EN ISO 10993-10: Biological evaluation of medical devices – Part 10: Articles 1, 2, 3, 4, 5, 6.2, 6.3, 6.4, 6.5, 7.5, Annex A, B.1, B.2, C, E, F) – skin irritation in a group of human volunteers.

**EXPERT OPINION:**
Test was performed by the Centre for Laboratory Testing, accredited by the Czech Accreditation Institute (Accredited Laboratory No.1206), Centre of Toxicology and Health Safety.

**CONCLUSION:**
**The material TM 3/20/73 is not regarded as a significant skin irritant.**

National Institute of Public Health
Centre of Toxicology and Health Safety
Šrobárova 49/48, 100 00 Praha 10
Czech Republic

Dagmar Jírová, M.D., Ph.D.
Centre of Toxicology and Health Safety

**ANNEX:**
Test Report No. 3/20/73 – Test report evaluation of human skin irritation

**National Institute of Public Health**
**Centre for Laboratory Testing**

**Laboratories of Toxicology**
**Šrobárova 49/48, 100 00 Prague 10**
**Tel.: +420 267082321 E–mail: hana.bendova@szu.cz**

L 1206

Laboratory No.1206, accredited by Czech Accreditation Institute
according to EN ISO/IEC 17025:2017

# Test Report No.3/20/73

**Customer: Nano Medical s.r.o.**

**Address: 5. května 1640/45, 140 00 Praha 4, Czech Republic**

**Reference No.: CTZB 187-3259/2020**

## Test Material

**Identification:**

**TM 3/20/73: AntiMicrobe Web R**

## Laboratory Tests

SOP 2/3 Tests for irritation and delayed-type hypersensitivity (EN ISO 10993–10: Biological evaluation of medical devices – Part 10, Articles 1, 2, 3, 4, 5, 6.2, 6.3, 6.4, 6.5, 7.5, Annex A, B.1, B.2, C, E, F) - skin irritation in a group of human volunteers

**Sample reception date:** 17.3.2020

**Date of study:** 24.3. - 27.3.2020

**Date of issue:** 30.3.2020

**Number of pages:** 7

**Authorized by Technical Manager: Hana Bendová, M.Sc., Ph.D.**

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The test results refer only to the sample as submitted by the sponsor and to the objectives of the study. This test report does not substitute for any other document or certification of the product. Without written approval of the testing laboratory this report should not be reproduced in other form than as a whole.

# TEST REPORT
# EVALUATION OF HUMAN SKIN IRRITATION

**Testing facility:** Laboratories of Toxicology, Centre for Laboratory Testing, National Institute of Public Health, Šrobárova 49/48, 100 00 Prague 10, Czech Republic.

**The test was carried out in compliance with: SOP 2/3** Test for irritation and delayed-type hypersensitivity (EN ISO 10993-10:2013: Part 10: articles 1, 2, 3, 4, 5, 6.2, 6.3, 6.4, 6.5, 7.5 Annex A, B.1, B.2, C, E, F)

**Aim of the study:** Assessment of the potential of the test material to produce dermal irritation.

## MATERIALS AND METHODS

### TEST MATERIAL (TM):

**TM 3/20/73: AntiMicrobe Web R**

**Sponsor:** **Nano Medical s.r.o.**
**5. května 1640/45**
**140 00 Praha 4**
**Czech Republic**

### PREPARATION OF MATERIALS FOR TESTING

- **Tested material**
  TM 3/20/73: applied directly on skin using gauze pad (2.5 x 2,5 cm).

### CONTROLS

- **Positive control (PC)**
  Sodium dodecyl sulfate (SDS) - 20% aqueous solution - applied directly on skin using gauze pad (0.4 ml).

### PARTICIPANTS IN THE STUDY

The selection of volunteers and the test methods complied with the Declaration of Helsinki (1964) and the International Ethical Guidelines for Biomedical Research Involving Human Subjects (CIOMS, 2002). The study was approved by the Ethical Review Committee of the National Institute of Public Health.

The volunteers were selected on the basis of inclusion and non-inclusion criteria and for this purpose filled in a special form. The volunteers were clearly informed regarding the nature of the study, timetable, constraints and possible risks. They



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gave their written informed consent before participation in the study was permitted. All the documentation is strictly confidential. 30 volunteers took part in the study.

**Table 1 – Demographic data**

| Subject Number | Subject Initials | Age | Gender |
|---|---|---|---|
| 1 | JG | 29 | F |
| 2 | VZ | 58 | F |
| 3 | JT | 19 | F |
| 4 | HP | 46 | M |
| 5 | JD | 67 | F |
| 6 | BH | 56 | F |
| 7 | KK | 54 | F |
| 8 | SM | 69 | F |
| 9 | JM | 48 | F |
| 10 | SD | 46 | M |
| 11 | SM | 45 | M |
| 12 | BI | 58 | M |
| 13 | BO | 27 | M |
| 14 | JM | 25 | M |
| 15 | JM | 45 | M |
| 16 | PJ | 29 | M |
| 17 | AT | 56 | M |
| 18 | DJ | 64 | M |
| 19 | ŠV | 32 | M |
| 20 | HI | 40 | F |
| 21 | LT | 56 | F |
| 22 | PM | 54 | F |
| 23 | JL | 58 | F |
| 24 | OD | 62 | F |
| 25 | DL | 69 | F |
| 26 | TJ | 55 | F |
| 27 | HZ | 55 | F |
| 28 | NE | 60 | F |
| 29 | HM | 53 | F |
| 30 | SM | 47 | F |



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**Test procedure**

- **Application of the test material**

The test material TM 3/20/73 was applied in occlusion on the upper outer arm (2.5 x 2.5 cm).

Occlusive: Hill Top Chambers (containing a gauze pad, diameter 1.8 cm), HILL TOP BIOLABS, USA

- **Application of the positive control**

The positive control - 20% SDS (0.4 ml) was applied on the upper outer arm.
Occlusive: Hill Top Chambers (containing a gauze pad, diameter 1.8 cm), HILL TOP BIOLABS, USA

- **Duration of exposure**

The patches were applied progressively starting with duration of 15 min and 30 min, and up to 1h, 2h, 3 h and 4h. The test substances were removed by rinsing and gentle swabbing.

- **Clinical observation and grading of skin reactions**

The reactions were assessed in the interval 0 h (immediately after patch removal), subsequently 1 - 2 h, 24 h, 48 h and 72 h after patch removal. Skin reactions were graded and recorded according to the grading given in Tab. 2.

**Table 2 – Human skin irritation test, grading scale**

| Description of response | Grading |
|---|---|
| No reaction | 0 |
| Weakly positive reaction (usually characterized by mild erythema and/or dryness across most of the treatment site) | 1 |
| Moderately positive reaction (usually distinct erythema or dryness, possibly spreading beyond of the treatment site) | 2 |
| Strongly positive reaction (strong and often spreading erythema with oedema and/or eschar formation) | 3 |

- **Data evaluation / interpretation**

The number of volunteers who developed a positive reaction after test material application (Tab. 3) and after positive control application (Tab. 4) was used for skin irritation evaluation.

The skin irritation potential hazard was determined by comparison of number of volunteers that produced skin reaction after test material application and number of volunteers that produced skin reaction after positive control application.

If the material produces a frequency of skin irritation in the test subjects which is substantially and significantly less than the positive control, it is not regarded as a significant skin irritant.

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If the material produces a frequency of skin irritation in the test subjects which is similar to, or grater than, the positive control, it is regarded as a significant skin irritant.

Fisher´s exact test was used for the statistical treatment of the results.

## RESULTS

The skin reactions are recorded in the Annex I.

## ASSESSMENT OF RESULTS

Skin reactions after application of the test material TM 3/20/73 were recorded for 0 of 30 volunteers. Skin reactions after application of the positive control were recorded for 30 of 30 volunteers. The Fisher´s exact test confirmed substantially and significantly lower frequency of the skin irritation in case of the test material application than in case of the positive control.

**The test material TM 3/20/73 is not regarded as a significant skin irritant.**

Test carried out by: Hana Bendová, M.Sc., Ph.D.

Principal investigator: Hana Bendová, M.Sc., Ph.D.

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## Annex I

**Table 3 - TM 3/20/73 - observation and grading of skin reactions**

| Volunteer No. | Time interval / Grading | | | | |
|---|---|---|---|---|---|
| | 0h grading | 1 - 2h grading | 24h grading | 48h grading | 72h grading |
| 1 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 |
| 4 | 0 | 0 | 0 | 0 | 0 |
| 5 | 0 | 0 | 0 | 0 | 0 |
| 6 | 0 | 0 | 0 | 0 | 0 |
| 7 | 0 | 0 | 0 | 0 | 0 |
| 8 | 0 | 0 | 0 | 0 | 0 |
| 9 | 0 | 0 | 0 | 0 | 0 |
| 10 | 0 | 0 | 0 | 0 | 0 |
| 11 | 0 | 0 | 0 | 0 | 0 |
| 12 | 0 | 0 | 0 | 0 | 0 |
| 13 | 0 | 0 | 0 | 0 | 0 |
| 14 | 0 | 0 | 0 | 0 | 0 |
| 15 | 0 | 0 | 0 | 0 | 0 |
| 16 | 0 | 0 | 0 | 0 | 0 |
| 17 | 0 | 0 | 0 | 0 | 0 |
| 18 | 0 | 0 | 0 | 0 | 0 |
| 19 | 0 | 0 | 0 | 0 | 0 |
| 20 | 0 | 0 | 0 | 0 | 0 |
| 21 | 0 | 0 | 0 | 0 | 0 |
| 22 | 0 | 0 | 0 | 0 | 0 |
| 23 | 0 | 0 | 0 | 0 | 0 |
| 24 | 0 | 0 | 0 | 0 | 0 |
| 25 | 0 | 0 | 0 | 0 | 0 |
| 26 | 0 | 0 | 0 | 0 | 0 |
| 27 | 0 | 0 | 0 | 0 | 0 |
| 28 | 0 | 0 | 0 | 0 | 0 |
| 29 | 0 | 0 | 0 | 0 | 0 |
| 30 | 0 | 0 | 0 | 0 | 0 |

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**Table 4 - Positive control - observation and grading of skin reactions**

| Volunteer No. | Time interval / Grading | | | | |
|---|---|---|---|---|---|
| | 0h grading | 1 - 2h grading | 24h grading | 48h grading | 72h grading |
| 1 | 0 | 0 | 1 | 1 | 1 |
| 2 | 2 | 3 | 3 | 3 | 3 |
| 3 | 0 | 0 | 1 | 1 | 1 |
| 4 | 2 | 3 | 3 | 3 | 3 |
| 5 | 1 | 1 | 2 | 3 | 3 |
| 6 | 1 | 2 | 3 | 3 | 3 |
| 7 | 1 | 1 | 2 | 3 | 3 |
| 8 | 1 | 2 | 2 | 2 | 1 |
| 9 | 1 | 2 | 3 | 3 | 3 |
| 10 | 2 | 3 | 3 | 3 | 3 |
| 11 | 0 | 0 | 1 | 1 | 1 |
| 12 | 0 | 0 | 1 | 1 | 1 |
| 13 | 1 | 2 | 3 | 3 | 3 |
| 14 | 1 | 2 | 3 | 3 | 3 |
| 15 | 0 | 0 | 1 | 1 | 1 |
| 16 | 0 | 1 | 1 | 1 | 1 |
| 17 | 1 | 2 | 3 | 3 | 3 |
| 18 | 1 | 1 | 2 | 3 | 3 |
| 19 | 0 | 0 | 1 | 1 | 1 |
| 20 | 1 | 1 | 2 | 3 | 3 |
| 21 | 1 | 1 | 1 | 1 | 1 |
| 22 | 1 | 1 | 2 | 2 | 2 |
| 23 | 0 | 1 | 2 | 3 | 3 |
| 24 | 2 | 3 | 3 | 3 | 3 |
| 25 | 0 | 1 | 2 | 3 | 3 |
| 26 | 0 | 1 | 1 | 1 | 1 |
| 27 | 1 | 1 | 1 | 1 | 1 |
| 28 | 1 | 1 | 1 | 1 | 1 |
| 29 | 0 | 1 | 1 | 1 | 1 |
| 30 | 1 | 2 | 2 | 2 | 2 |

----------end of report----------

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