



Test Report

TBK-2020-010270

Metal specimen (NWK A-BMV)

Antibacterial activity test

President of KTR

Kwon Oh-jung

Test outline

Test Title : Antibacterial activity test
Report Number : TBK-2020-010270
Test Method : Referencing ISO 22196 : 2011

Sponsor

Name: NAE WOI KOREA.,LTD.
Address: #501 W-City, 9-22, 255Pangyo-ro, Bundang-gu, Seongnam-si, Gyeonggi-do, Korea
Representative: Yang,CheolHo

Test Facility

Name: Korean Testing & Research Institute
Address: 98, Gyoyukwon-ro, Gwacheon-si, Gyeonggi-do, Korea

KO Eun Ok 고은옥

Ko, Eun-Ok, B.S.

Study Director

Medical device-Bio Research Institute, KTR

Cho Jin Sik 조진식

Cho, Jin-Sik, M.S.

Technical Manager

Medical device-Bio Research Institute, KTR

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Date

2021-01-21

Date

This report is presented for the article that was submitted by the sponsor.

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CONTENTS

| | |
|--|---|
| 1. Summary | 1 |
| 1.1. Test Schedule | 1 |
| 1.2. Picture of the test article | 1 |
| 2. Equipment & materials | 2 |
| 2.1. Test Equipment | 2 |
| 2.2. Test Material | 2 |
| 3. Test method | 3 |
| 3.1. Test Method | 3 |
| 3.2. Calculation of Result | 3 |
| 3.3 Test requirements | 4 |
| 4. Result | 5 |
| 4.1. Antibacterial activity test against <i>S. typhimurium</i> | 5 |
| 4.2. Antibacterial activity test against VRE | 5 |
| 5. Conclusion | 6 |
| 6. References | 7 |
| 7. Tables | 8 |
| 8. Attachment | 9 |
| 8.1. Pictures of the test results | 9 |

Table contents

| | |
|--|---|
| Table 1. Test result against <i>S. typhimurium</i> | 5 |
| Table 2. Test result against VRE | 5 |
| Table 3. Summary of antibacterial activity test result | 6 |

1. Summary

This test was performed to assess the antimicrobial activity of the test article provided by the sponsor according to ISO 22196 : 2011.

S. typhimurium (Gram negative) and VRE (Vancomycin-Resistant *Enterococcus faecalis*) (Gram positive) were used as the test strains as requested by the client.

After inoculating the test strains to the specimen, every test specimen was incubated at a temperature of $(35 \pm 1) ^\circ\text{C}$ with more than 90 % of relative humidity for (24 ± 1) hours.

And then the number of viable cells was measured by recovering the strains from each specimen and the antibacterial activity was measured by comparing to the number of viable cells in the control group (untreated test pieces after 24 hours).

The antibacterial activity of the test article [Metal specimen (NWK A-BMV)] under these test conditions, showed antibacterial activity of 4.7 and 3.3 against *S. typhimurium* and VRE respectively.

1.1. Test Schedule

The total period of test 31 December 2020 to 21 January 2021

1.1. Picture of the test article



TBK-2020-010270

2. Equipment & materials

2.1. Equipment

| | |
|-------------------|--|
| Auto clave | (core tech, Korea) |
| Dry oven | (Jisico, Korea) |
| Water bath | (Polyscience, USA) |
| Incubator | (Mettler, Germany) |
| pH meter | (Thermo Orion, USA) |
| Stop watch | (Time Art, Japan) |
| Vortex mixer | (ThermoFisher, USA) |
| Container | (Iwaki Pyrex, Japan) |
| Sterile pipette | (FALCON, USA) |
| Petri dish | (SPL, Korea) |
| Volumetric flask | (Myung Sung, Korea) |
| Mechanical shaker | (Jisico, Korea) |
| Clean bench | (Su Gong Yang Hang, Korea) |
| Colony counter | (Dukwoo Science, Korea) |
| Film | (sterilize poly bag for Stomacher /interscience, France) |

2.2. Test materials

2.2.1. Test organism

Salmonella typhimurium ATCC 13311

VRE (Vancomycin-Resistant *Enterococcus faecalis*) ATCC 51299

2.2.2. Media and test reagents

Nutrient broth (Difco, USA)

Nutrient agar (Difco, USA)

Plate count agar (Difco, USA)

SCDLP broth (Eiken, Japan)

Phosphate-buffered physiological saline

3. Test method

3.1. Test method

3.1.1. Pre-incubation of test bacteria

The reserved test strains were inoculated on slant media and incubated at a temperature of $(35 \pm 1) ^\circ\text{C}$ for 16 hours to 20 hours.

3.1.2. Preparation of test inoculum

The incubated test strain was diluted with 1/500 NB(Nutrient Broth) to make initial bacterial number within the range of $(2.5 \sim 10) \times 10^5$, and that was used as inoculation solution.

3.1.3. Preparation of test piece

The flat portion of test articles and controls were prepared in the size of (50 ± 2) mm and the surface of all test specimen were sterilized by testing a UV lamp before using then for test. The film of section 2.1 was used for the untreated test group and the covering film.

3.1.4. Inoculation with test inoculum and incubation

0.4 mL of bacterial inoculum solution was added onto specimen in a petri dish, and a film was placed on the inoculated surface to spread bacterial inoculum evenly. The Petri dish was covered and incubated at a temperature of $(35 \pm 1) ^\circ\text{C}$ with more than 90 % of relative humidity for (24 ± 1) hours.

3.1.5. Recovery of test and measurement

Using 10 mL of SCDLP broth, the bacteria were collected from the untreated test group immediately (after the inoculation), the treated test group (after 24 hours of incubation) and untreated test group(after 24 hours of incubation).

After incubating the collected bacteria by using the agar plate culture method at $(35 \pm 1) ^\circ\text{C}$ for 40 hours to $(40 \sim 48)$ hours, the number of viable bacteria was measured according to the calculation of section 3.2.1.

3.2. Calculation of result

3.2.1. Viable cell count

$$N = (C \times D \times V)/A$$

N : number of viable bacteria (per 1 cm^2 of test piece)

C : count of colonies (Average count of colonies of two petri dishes adopted)

D : dilution factor (that of dilution dispensed into petri dishes adopted)

V : Volume of SCDLP broth used for wash-out (mL)

A : surface area of covering film (cm^2)

3.2.2. Antibacterial activity calculation

$$R = (U_t - U_o) - (A_t - U_o) = U_t - A_t$$

R : antibacterial activity

U_o : average of the number of viable cells of bacteria immediately after inoculation on the untreated test piece

U_t : average of the number of viable cells of bacteria on the untreated test piece after 24 h

A_t : average of the number of viable cells of bacteria on the antimicrobial test piece after 24 h

3.3. Test requirements

3.3.1. Log viable cell count

$$(L_{max} - L_{min}) / (L_{mean}) \leq 0.2$$

L_{max} : maximum value of Log viable cell count

L_{min} : minimum value of Log viable cell count

L_{mean} : mean value of Log viable cell count of 3 test specimens

3.3.2. Number of viable cells of untreated test group right after inoculation

The average of the number of viable bacterial immediately after inoculation on the untreated test piece shall be within the range of 6.2×10^3 to 2.5×10^4 CFU/cm².

3.3.3. Number of viable cells of untreated test group after 24 hours of incubation

The number of viable bacteria on untreated test piece after 24 hours shall be not less than 62 CFU/cm² (If film is used instead of untreated test group, viable cell count of 3 test specimens shall be not less than 6.2×10^2 CFU/cm²).

4. Results

4.1. Antibacterial activity test against *S. typhimurium*

The average of logarithmic value of the number of viable cells of bacteria immediately after inoculation on the untreated feat piece (U_o) was 4.0. The average of logarithmic value of the number of viable cells of bacteria on the untreated test piece after 24 h (U_t) was 4.5. The average of logarithmic value of the number of viable cells of bacteria on the antimicrobial test piece after 24 h (A_t) was - 0.2. The antibacterial activity (R) was calculated as 4.7 according to the formula in paragraph 3.2.2.

Table 1. Test result against *S. typhimurium* (Table 1)

| Number of repeats | Initial | | untreated test piece (after 24 hours) | | antimicrobial test piece (after 24 hours) | |
|-------------------|-----------------------|-------------------|--|-------------------|--|-------------------|
| | CFU/cm ² | logarithmic value | CFU/cm ² | logarithmic value | CFU/cm ² | logarithmic value |
| 1 | 1.2 x 10 ⁴ | 4.08 | 3.0 x 10 ⁴ | 4.48 | < 0.63 | - 0.20 |
| 2 | 1.2 x 10 ⁴ | 4.08 | 3.5 x 10 ⁴ | 4.54 | < 0.63 | - 0.20 |
| 3 | 1.2 x 10 ⁴ | 4.08 | 4.1 x 10 ⁴ | 4.61 | < 0.63 | - 0.20 |

4.2. Antibacterial activity test against VRE

The average of logarithmic value of the number of viable cells of bacteria immediately after inoculation on the untreated feat piece (U_o) was 4.3. The average of logarithmic value of the number of viable cells of bacteria on the untreated test piece after 24 h (U_t) was 4.5. The average of logarithmic value of the number of viable cells of bacteria on the antimicrobial test piece after 24 h (A_t) was 1.2. The antibacterial activity (R) was calculated as 3.3 according to the formula in paragraph 3.2.2.

Table 2. Test result against VRE (Table 2)

| Number of repeats | Initial | | untreated test piece (after 24 hours) | | antimicrobial test piece (after 24 hours) | |
|-------------------|-----------------------|-------------------|--|-------------------|--|-------------------|
| | CFU/cm ² | logarithmic value | CFU/cm ² | logarithmic value | CFU/cm ² | logarithmic value |
| 1 | 2.0 x 10 ⁴ | 4.30 | 2.9 x 10 ⁴ | 4.46 | 20 | 1.30 |
| 2 | 2.1 x 10 ⁴ | 4.32 | 3.1 x 10 ⁴ | 4.49 | 16 | 1.20 |
| 3 | 1.9 x 10 ⁴ | 4.28 | 3.7 x 10 ⁴ | 4.57 | 13 | 1.11 |

Table 3. Summary of result of Antibacterial activity test

(Unit : log value)

| Test strain | U _o | U _t | A _t | R |
|-----------------------|----------------|----------------|----------------|-----|
| <i>S. typhimurium</i> | 4.0 | 4.5 | - 0.2 | 4.7 |
| VRE | 4.3 | 4.5 | 1.2 | 3.3 |

$$R = (U_t - U_o) - (A_t - U_o) = U_t - A_t$$

R : antibacterial activity

U_o : average of logarithm numbers of viable bacteria immediately after inoculation on untreated test pieces

U_t : average of logarithm numbers of viable bacteria after inoculation on untreated test pieces after 24 h

A_t : average of logarithm numbers of viable bacteria after inoculation on Antibacterial activity test piece after 24 h

5. Conclusion

This test was performed to assess the antimicrobial activity of the test article provided by the sponsor according to ISO 22196 : 2011.

After inoculating the test strains to the specimen, every test specimen was incubated at $(35 \pm 1) ^\circ\text{C}$ with more than 90 % of relative humidity for (24 ± 1) hour. And then the number of viable cells was measured by recovering the strains from each specimens and the antibacterial activity was measured by comparing the number of viable cells in the untreated test group.

The antibacterial activity¹⁾ of the test article [Metal specimen (NWK A-BMV)] under these test conditions showed the activity of 4.7 and 3.3 against *S. typhimurium* and VRE respectively.

The effectiveness of this test was validated because the test requirements of the test was satisfied with the logarithm number of viable bacteria and untreated test pieces number of viable bacteria which were defined in ISO 22196 : 2011.

¹⁾* Interpretation of results

| Antibacterial activity | Antibacterial effect |
|------------------------|----------------------|
| ≥ 1 | ≥ 90.00 % |
| ≥ 2 | ≥ 99.00 % |
| ≥ 3 | ≥ 99.90 % |
| ≥ 4 | ≥ 99.99 % |
| ≥ 5 | ≥ 99.999 % |

6. References

- 6.1. ISO 22196 : 2011, Measurement of antibacterial activity on plastics and other non-porous surfaces
- 6.2. JIS Z 2801 : 2012, Antibacterial products–Test for antibacterial activity and efficacy
- 3.1. BS EN 1276 : 2019, Chemical disinfectants and antiseptics–Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas – test method and requirements (phase 2, step 1)
- 6.3. ASTM E 2180–01, Standard test method for determining the activity of incorporated antimicrobial agents in polymeric or hydrophobic Materials
- 6.4. KS K 0693 : 2011, Test method for antibacterial activity of textile materials

7. Attachment

7.1. Picture of test result



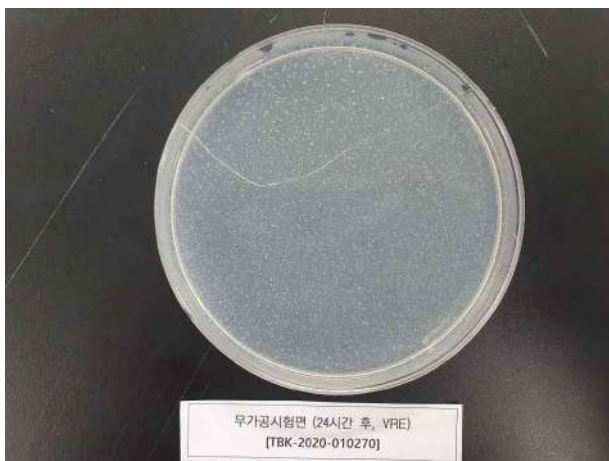
무가공시험편 (24시간 후, *S. typhimurium*)
[TBK-2020-010270]

Untreated test group
(after 24 hours, *S. typhimurium*)
[TBK-2020-010270]



항균가공시험편 (24시간 후, *S. typhimurium*)
[TBK-2020-010270]

Antibacterial activity test group
(after 24 hours, *S. typhimurium*)
[TBK-2020-010270]



무가공시험편 (24시간 후, VRE)
[TBK-2020-010270]

Untreated test group
(after 24 hours, VRE)
[TBK-2020-010270]



항균가공시험편 (24시간 후, VRE)
[TBK-2020-010270]

Antibacterial activity test group
(after 24 hours, VRE)
[TBK-2020-010270]

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