Dr. Christian Jursch
**Eurovir Hygiene-Labor GmbH**
Im Biotechnologiepark TGZ I
D-14943 Luckenwalde



# Testing the virucidal activity of *" Liquid Guard"*

Examination of test surfaces equipped with a virucidal active coating using a praxis-near carrier test system following the RKI-Richtlinie (1995) as well as ISO 21702:2019 against the *Transmissible Gastroenteritis Virus (TGEV-Coronavirus)* - Test run S1 dated 11./12.03.2020

Short report: screening test S2

by
PD Dr. Olaf Thraenhart and Dr. Christian Jursch

| | |
|---|---|
| Test period: | in March 2020 |
| Principal: | Nano-Care Deutschland AG<br>Alfred Nobel-Straße 10<br>D-66793 Saarwellingen, Germany |

***Principal:*** Nano-Care UK (Signo-Nanocare UK Ltd)
PO Box 225, Oswestry, Shropshire, SY10 1DL, UK +44 1691 654 282

***Products:***

- Test surfaces: *Leneta®* foil, with the dimensions of 1,6 cm x 6 cm
- 1. test item: test surfaces coated on one side with *Liquid Guard* (containing the active component[s])
- 2. test item: uncoated test surfaces (or coated w/o the active component[s])

***Test parameter:***

- Test conditions: T = 25 °C and 90 % r.LF
- Protein load: no additional protein load; the virus material (cell culture supernatant) was spread onto the surface(s) w/o any further manipulation/alteration
- Volume to square ratio: 25 µL/cm²
- Virus suspension covered with foil (LDPE, 50 µm) with the dimensions 1,2 x 5 cm (6 cm²)
- Incubation: 1h, 8h and 24h in a climate chamber KBF 115 (Fa Binder)

***Test system:***

- Transmissible Gastroenteritis Virus of Swine (TGEV-Coronavirus); strain: Toyama 36 [used in test as the model virus for SARS-CoV]
  (Origin: Virusbank of the Friedrich Löffler-Institute, Insel Riems, Germany)
- ST75/2 cells (foetal testis cells of swine)
  (Origin: Robert Koch-Institute, Berlin, Germany)

***Test procedure:***

- The test was performed following a. RKI-Richtlinie (1995) as well as b. ISO 21702:2019
- Test principle: quantitative virucidal carrier test at T = 25 °C and 90 % r.LF (climate chamber)
- the test was performed w/o (additional) protein load

***Tab. 1: Product samples tested***

| No. | Product (s) | Storage conditions [1] |
|---|---|---|
| #1 | Test item / coated with *Liquid Guard*<br>(containing the virucidal active component(s) / „test sample“) | at RT |
| #2 | Test item / uncoated<br>(or coated w/o the virucidal active component(s) / „control sample“) | at RT |

[1] = *access limited*

Dr. Christian Jursch
**Eurovir Hygiene-Labor GmbH**
Im Biotechnologiepark TGZ I
D-14943 Luckenwalde



***Test results:***

***Observations:***

- The test surfaces were largely wetable by the aqueous virus suspension; thus, a more or less uniform liquid film could be produced by using glass spatulas.
- After covering the virus with the LDPE foil, the virus material remained stable as a film over the entire observation period and did not dry out. However, a volume reduction was recorded.

***Tab. 2.1: Virus control*** *(Virus titration by limiting dilution)*

| Sample | VK-1a | VK-1b | VK-2a | VK-2b | VK-3a | VK-3b |
|---|---|---|---|---|---|---|
| | Virus control / 1 h | | Virus control / 8 h | | Virus control / 24 h | |
| Titer/Test vol. ($lg\ ID_{50}$) | 4,2 | 4,8 | 4,05 | 3,9 | 2,25 | 2,85 |
| **av. virus titer ± K (95%) [1]** | **5,50 ± 0,37 / 1 mL** | | **4,98 ± 0,35 / 1 mL** | | **3,55 ± 0,37 / 1 mL** | |

[1] = Calculation of the virus titer and its 95% confidence interval according to EN14476

***Tab. 2.2: Virus inactivation*** *(Virus titration by limiting dilution)*

| Sample | In-1a | In-1b | In-2a | In-2b | In-3a | In-3b |
|---|---|---|---|---|---|---|
| | Inactivation / 1 h | | Inactivation / 8 h | | Inactivation / 24 h | |
| Titer/Test vol. ($lg\ ID_{50}$) | 3,6 | 3,45 | 1,35 | 1,2 | ≤ 0,30 | ≤ 0,30 |
| av. virus titer ± K (95%) [1] | 4,53 ± 0,22 / mL | | 2,28 ± 0,29 / mL | | ≤ 1,30 / mL | |
| **Reduction [2] ($lg\ ID_{50}$ ± K [95%])** | **0,97 ± 0,43** | | **2,70 ± 0,46** | | **≥ 2,25 ± 0,37** | |

[1] = Calculation of the virus titer and its 95% confidence interval according to EN14476
[2] = Virus reduction: lg $ID_{50}$ of virus input (virus control) minus lg $ID_{50}$ of sample (at the given time point)

***Virus inactivation:*** *(cf. Tab. 2)*

- When the virus material is distributed onto a surface a certain virus titer reduction could be observed with almost all viruses. This is driven by time and do also occur without any other influence. This is also true for the test virus used in the present testing. After presentation over 8 h and 24 h on the test surface a titer reduction of 0,5 Log was evident after 8 h and about 2 Log after 24 h (cf. tab. 2.1). It should be noted, however, that this reduction can be judged as very low when compared to 1). the general tenacity of coronaviruses and b). other viruses (even non-enveloped viruses).
- In order to assess the virus inactivating capacity of the coating under test as a single factor an individual virus input control was analysed at each time point tested. With the amount of input virus at a given time point (cf. tab. 2.1) and with the correspondent amount of remaining test virus (cf. tab. 2.2) the virus reduction factor can be determined.
- After the incubation time was due and under the test conditions specified above the virus reduction factor associated with the coating containing the active component amounted to RF = 0,97 ± 0,43 after 1 h, to RF = 2,70 ± 0,46 after 8 h and to RF ≥ 2,25 ± 0,37 after 24 h (cf. Tab. 2.2). It should be noted that after 24 h no residual test virus was detectable.

Dr. Christian Jursch
**Eurovir Hygiene-Labor GmbH**
Im Biotechnologiepark TGZ I
D-14943 Luckenwalde



***Conclusions:***

- The virus film applied on the test items and covered with the LDPE-foil was stable over the entire observation period. This means that the virus film remained in the liquid state even at the end of the longest exposure time (24 h) and was not dried. Thus, a continuous contact between the virus material and the surface of the test carrier was ensured all over the observation period and a distribution of the virus material in the liquid phase driven by diffusion was given.
- After t = 1 h a virus reduction of 0,97 Log was recorded (corresponding to 90 % of inactivation) and after t = 8 h the virus reduction amounted to RF = 2,7 (corresponding to 99,8 % of inactivation). Due to technical reasons demonstration of the virus reduction was limited to RF $\geq$ 2,25 after 24 h.
- The data obtained allow the conclusion that there is a virus reduction that can be attributed to the coating containing the active component(s). With the present testing a good virus inactivating activity of the virucidal coating under test was demonstrated against the TGEV-Coronavirus (as the model virus for the SARS-CoV).
- It should also be mentioned that the conditions of ISO 21702 provide for a higher incubation temperature than that used in S1 (25 vs. 21 ° C).
- The virus reduction obtained with t = 8 h suggests that at the incubation time t = 24 h a higher virus reduction is evident than could be demonstrated with the endpoint titration method. Here, virus titer determination using the *Large Volume Plating (LVP)* can possibly provide an improved statement.

Luckenwalde, 20th of March 2020

Dr. Ch. Jursch
(GF und Laborleiter Eurovir)

Dr. Christian Jursch
**Eurovir Hygiene-Labor GmbH**
Im Biotechnologiepark TGZ I
D-14943 Luckenwalde

Eurovir® Hygiene-Labor
Antivirale Validierung & Rabies

# Testing the virucidal activity of *" Liquid Guard"*

Examination of test surfaces equipped with a virucidal active coating using a praxis-near carrier test system following the RKI-Richtlinie (1995) as well as JIS Z 2801 (2010) against *Influenza A Virus (H1N1)* - Test run S1 dated 20./21.01.2020

Short report: screening test S1

by
PD Dr. Olaf Thraenhart and Dr. Christian Jursch

| | |
|---|---|
| Test period: | in January 2020 |
| Principal: | Nano-Care Deutschland AG<br>Alfred Nobel-Straße 10<br>D-66793 Saarwellingen, Germany |

Dr. Christian Jursch
**Eurovir Hygiene-Labor GmbH**
Im Biotechnologiepark TGZ I
D-14943 Luckenwalde



***Principal:*** Nano-Care UK (Signo-Nanocare UK Ltd)
PO Box 225, Oswestry, Shropshire, SY10 1DL, UK +44 1691 654 282

***Products:***

- Test surfaces: *Leneta®* foil, with the dimensions of 1,6 cm x 6 cm
- 1. test item: test surfaces coated on one side with *Liquid Guard* (containing the active component[s])
- 2. test item: uncoated test surfaces (or coated w/o the active component[s])

***Test parameter:***

- Test conditions: T = 21 °C and 60 % r.LF
- Protein load: no additional protein load; the virus material (cell culture supernatant) was spread onto the surface(s) w/o any further manipulation/alteration
- Volume to square ratio: 20 µL/cm²
- Virus suspension covered with foil (LDPE, 50 µm) with the dimensions 1,2 x 5 cm (6 cm²)
- Incubation: 1h, 8h and 24h in a climate chamber KBF 115 (Fa Binder)

***Test system:***

- Influenza A Virus; H1N1; strain: New Caledonia
  (Origin: Chiron Behring, Marburg, Germany)
- MDCK-cells (kidney cells from African green monkey [Cercopethecus aethiops])
  (Origin: Robert Koch-Institut, Berlin, Germany)

***Test procedure:***

- The test was performed following a. RKI-Richtlinie (1995) as well as b. JIS Z 2801 (2010)
- Test principle: quantitative virucidal carrier test at T = 21 °C and 60 % r.LF (climate chamber)
- the test was performed w/o (additional) protein load

***Tab. 1:* Product samples tested** *(as received at 13.01.2020)*

| No. | Product (s) | Storage conditions [1] |
|---|---|---|
| #1 | Test item / coated with *Liquid Guard*<br>(containing the virucidal active component(s) / „test sample“) | at RT |
| #2 | Test item / uncoated<br>(or coated w/o the virucidal active component(s) / „control sample“) | at RT |

[1] = *access limited to the personnel of Eurovir*

***Test results:***

***Observations:***

- The test surfaces were largely wetable by the aqueous virus suspension; thus, a more or less uniform liquid film could be produced by using glass spatulas.
- After covering the virus with the LDPE foil, the virus material remained stable as a film over the entire observation period and did not dry out. However, a volume reduction was recorded.

***Tab. 2.1: Virus control*** *(Virus titration by limiting dilution)*

| Sample | VK-1a | VK-1b | VK-2a | VK-2b | VK-3a | VK-3b |
|---|---|---|---|---|---|---|
| | Virus control / 1 h | | Virus control / 8 h | | Virus control / 24 h | |
| Titer/Test vol. ($lg\ ID_{50}$) | 3,15 | 3,3 | 3,45 | 3,15 | 2,7 | 3,15 |
| **av. virus titer ± K (95%) [1]** | **3,23 ± 0,36 / 100 µL** | | **3,30 ± 0,33 / 100 µL** | | **2,93 ± 0,34 / 100 µL** | |

[1] = Calculation of the virus titer and its 95% confidence interval according to EN14476

***Tab. 2.2: Virus inactivation*** *(Virus titration by limiting dilution)*

| Sample | In-1a | In-1b | In-2a | In-2b | In-3a | In-3b |
|---|---|---|---|---|---|---|
| | Inactivation / 1 h | | Inactivation / 8 h | | Inactivation / 24 h | |
| Titer/Test vol. ($lg\ ID_{50}$) | 3,45 | 3,15 | 2,4 | 2,4 | 1,2 | 1,2 |
| av. virus titer ± K (95%) [1] | 3,30 ± 0,32 | | 2,40 ± 0,29 | | 1,20 ± 0,33 | |
| **Reduction [2] ($lg\ ID_{50}$ ± K [95%])** | **-0,07 ± 0,48** | | **0,90 ± 0,44** | | **1,73 ± 0,47** | |

[1] = Calculation of the virus titer and its 95% confidence interval according to EN14476
[2] = Virus reduction: lg $ID_{50}$ of virus input (virus control) minus lg $ID_{50}$ of sample (at the given time point)

***Virus inactivation:*** *(cf. Tab. 2)*

- When the virus material is distributed onto a surface a certain virus titer reduction could be observed with almost all viruses. This is driven by time and do also occur without any other influence. This is also true for the test virus used in the present testing. After presentation over 24 h on the test surface a titer reduction of 0,3 Log was evident (cf. tab. 2.1). It should be noted, however, that this reduction can be judged as very low when compared to 1). the general tenacity of influenza virus and b). other viruses (even non-enveloped viruses).
- In order to assess the virus inactivating capacity of the coating under test as a single factor an individual virus input control was analysed at each time point tested. With the amount of input virus at a given time point (cf. tab. 2.1) and with the correspondent amount of remaining test virus (cf. tab. 2.2) the virus reduction factor can be determined.
- After the incubation time was due and under the test conditions specified above the virus reduction factor associated with the coating containing the active component amounted to RF = -0,07 ± 0,48 after 1 h, to RF = 0,90 ± 0,44 after 8 h and to RF = 1,73 ± 0,47 after 24 h (cf. Tab. 2.2).

Dr. Christian Jursch
**Eurovir Hygiene-Labor GmbH**
Im Biotechnologiepark TGZ I
D-14943 Luckenwalde



***Conclusions:***

- The virus film applied on the test items and covered with the LDPE-foil was stable over the entire observation period. This means that the virus film remained in the liquid state even at the end of the longest exposure time (24 h) and was not dried. Thus, a continuous contact between the virus material and the surface of the test carrier was ensured all over the observation period and a distribution of the virus material in the liquid phase driven by diffusion was given.
- The data obtained allow the conclusion that there is a virus reduction that can be attributed to the coating containing the active component(s).
- The virus reduction rate progresses rather slowly over the observation period. No virus inactivation was detectable after a contact time of 1 hour and after 8 hours the virus reduction was approximately 1 Log (corresponding to a virus reduction of approximately 90%). After 24 hours virus reduction reached approximately 2 Log (corresponding to a reduction of approximately 99%).
- The observed virus-inactivating effect of the coating (containing the active component[s]) was determined using the *influenza A virus* as the test virus. This virus is in general considered to be inactivated easily, even when compared with other enveloped virus. This means that the observed virus inactivation capacity of the tested coating, as obtained with *influenza A virus,* cannot be transferred necessarily to other viruses. This also applies to other enveloped viruses.

Luckenwalde, 4th of March 2020

Dr. Ch. Jursch
(GF and Laboratory manager of Eurovir)

| | |
|---|---|
| Work Order | 3109.4W |
| Setup-Code | 180612-10290-2801-01 |

# Test Report

# JIS Z 2801:2012 (Mod)

## Antimicrobial products – Test for antimicrobial activity and Effectiveness

## Test Object:

*Coated Leneta-Foil vs. Aspergillus niger DSM 1988*

# Report on Findings

**Client:**
**Address:** Nano-Care UK (Signo-Nanocare UK Ltd)
PO Box 225, Oswestry, Shropshire, SY10 1DL, UK +44 1691 654 282

**Work order no.:** 3109.4W

**Test object:** Coated Leneta-Foil vs. Aspergillus niger DSM 1988

**Sample description:** Coated Leneta Foil

**Date of receipt of sample:** 14.09.2018

**Type of test:** JIS Z 2801:2012 Antimicrobial products – Test for antimicrobial activity and efficacy

**Test Germ:** Aspergillus niger DSM 1988

**Test laboratory:** QualityLabs BT GmbH

**Address:** Neumeyerstrasse 46a
90411 Nuremberg, Germany

**Setup-Code:** 180612-10290-2801-01

**Sample material:** Leneta-Foil

**No. of pages in report:** 7

**Report on findings to the client:**
**Place and date of preparation:** Nuremberg, 14.9.2018
**Recipient:** Nano-Care Deutschland AG

**Laboratory Director:** _____
Harald Gerauer, Laboratory Director
QualityLabs BT GmbH

**Released:** _____
Markus Zehe, Managing Director
QualityLabs BT GmbH

| | |
|---|---|
| Work Order | 3109.4W |
| Setup-Code | 180612-10290-2801-01 |

## Declaration on Quality Assurance

This investigation was performed and supervised according to the standard operating procedure “SOP zu JIS Z 2801:2012 (Mod)" by QualityLabs BT GmbH. The laboratory and process are continually monitored by independent, external authorities, as well as by internal audits.

### Archiving

A copy of the test report, a protocol of the measurement as well as the accompanying correspondence and business records are archived by QualityLabs BT GmbH. The retention period is at least 10 years.

## Test description

Anti-bacterial activity is determined in accordance with a modified version of JIS Z 2801:2012.

During the test, a thin liquid-film containing the bacteria ($1.25 \times 10^4$ CFU / $cm^2$) is applied directly to the test sample (Standard: 5 cm x 5 cm). To avoid desiccation a foil (Standard: 4cm x 4cm, Stomacher Bags) is applied. Immediately after inoculation, the bacteria from the reference sample are separated from the sample and the enveloping foil surfaces using ultrasound and vortex devices and the number of viable germs (CFU – colony-forming units) is determined ($t_0$ value). A further set of reference samples and samples given anti-microbial treatment is incubated with bacteria in a liquid-film and the enveloping foil in a damp environment at 37°C. After 24 hours, the bacteria are separated from the sample surfaces using ultrasound and vortex devices and the number of viable germs is determined ($t_{24}$ value).

## Assessment of antimicrobial activity

A logarithmic germ reduction of **≥ 3 log scales** of the antimicrobial sample in comparison to the respective reference is used as assessment criterion to pass the antimicrobial test.

| Germ reduktion [log scales] | Antibacterial activity |
|---|---|
| < 3 | Not sufficient antimicrobial activity |
| ≥ 3 | Sufficient antimicrobial activity |

## References to Testconditions

| Testconditions | | |
|---|---|---|
| Sample size | 25 | $cm^2$ |
| Foil size | 16 | $cm^2$ |
| Volume Inoculum | 400 | µl |
| Sample cleaning | Isopropanol | - |

## References to deviations, preincubations, special test conditions

NONE

| | |
|---|---|
| Work Order | 3109.4 |
| Setup-Code | 180612-10290-2801-01 |

# Test Results

| | Sample Name | Sample Code | $t_0$ (cells/cm$^2$) | | | $t_{24}$ (cells/cm$^2$) | | | Reduction [%] | Log Reduction |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Leneta-Foil P121-10 (Reference) | 102900806180001 | 2,5 x $10^4$ | 2,7 x $10^4$ | 2,6 x $10^4$ | 2,1 x $10^4$ | 2,5 x $10^4$ | 2,7 x $10^4$ | - | - |
| 2 | Liquid Guard clean + primer + wipe | 102900806180002 | | | | 6,4 x $10^3$ | 6,1 x $10^3$ | 3,5 x $10^3$ | **78,12** | **0,66** |
| 3 | Liquid Guard pro + wipe | 102900806180003 | | | | 1,9 x $10^4$ | 6,1 x $10^3$ | 2,5 x $10^4$ | **0,02** | **0,02** |

*see “Interpretation of Results”, page 6

| | |
|---|---|
| **Test strain** | *Aspergillus niger DSM1988* |
| **Initial cell count inoculum / cm$^2$** | **1.25 x $10^4$** |
| **Initials of the editor** | OS |
| **Measurement ended on** | 17.07.2018 |

| | |
|---|---|
| Work Order | 3109.4 |
| Setup-Code | 180612-10290-2801-01 |

## Comments on test objects

NONE

## Interpretation of the results based on the measurements

NONE

**Editor:** Mr. Shendi ____ **Crosschecked:** Mr.Zehe ____

## References

JIS Z 2801:2012 Antimicrobial products – Test for antimicrobial activity and efficacy

| | |
|---|---|
| Work Order | 3250 |
| Setup-Code | 181218-10290-2801-01 |

# Test Report

# JIS Z 2801:2012 (Mod)

## Antimicrobial products – Test for antimicrobial activity and efficacy

## Test Object:

*Coated Leneta-Foil versus Clostridioides difficile DSM 27543*

| | |
|---|---|
| Work Order | 3250 |
| Setup-Code | 181218-10290-2801-01 |

# Report on Findings

**Client:** Nano-Care UK (Signo-Nanocare UK Ltd)
**Address:** PO Box 225, Oswestry, Shropshire, SY10 1DL, UK +44 1691 654 282

**Work order no.:** 3250

**Test object:** Coated Leneta-Fiol versus *Clostridioides difficile* DSM 27543

**Sample description:** coated foil

**Date of receipt of sample:** 2018-Oct-22

**Type of test:** JIS Z 2801:2012 Antimicrobial products – Test for antimicrobial activity and efficacy

**Test Germ:** *Clostridioides difficile* DSM 27543

**Test laboratory:** QualityLabs BT GmbH

**Address:** Neumeyerstrasse 46a
90411 Nuremberg, Germany

**Setup-Code:** 181218-10290-2801-01

**Sample material:** n.b.

**No. of pages in report:** 7

**Report on findings to the client:** **Place and date of preparation:** Nuremberg, 2018-Dez-18
**Recipient:** Nano-Care Deutschland GmbH

**Laboratory Director:** _____
Harald Gerauer, Laboratory Director
QualityLabs BT GmbH

**Released:** _____
Markus Zehe, Managing Director
QualityLabs BT GmbH

| | |
|---|---|
| Work Order | 3250 |
| Setup-Code | 181218-10290-2801-01 |

## Declaration on Quality Assurance

This investigation was performed and supervised according to the standard operating procedure "SOP zu JIS Z 2801:2012 (Mod)" by QualityLabs BT GmbH. The laboratory and process are continually monitored by independent, external authorities, as well as by internal audits.

### Archiving

A copy of the test report, a protocol of the measurement as well as the accompanying correspondence and business records are archived by QualityLabs BT GmbH. The retention period is at least 10 years.

## Test description

Anti-bacterial activity is determined in accordance with a modified version of JIS Z 2801:2012.

During the test, a thin liquid-film containing the bacteria ($1.25 \times 10^4$ CFU / $cm^2$) is applied directly to the test sample (Standard: 5 cm x 5 cm). To avoid desiccation a foil (Standard: 4cm x 4cm, Stomacher Bags) is applied. Immediately after inoculation, the bacteria from the reference sample are separated from the sample and the enveloping foil surfaces using ultrasound and vortex devices and the number of viable germs (CFU – colony-forming units) is determined ($t_0$ value). A further set of reference samples and samples given anti-microbial treatment is incubated with bacteria in a liquid-film and the enveloping foil in a damp environment at 37°C. After 24 hours, the bacteria are separated from the sample surfaces using ultrasound and vortex devices and the number of viable germs is determined ($t_{24}$ value).

## Assessment of antimicrobial activity

A logarithmic germ reduction of **≥ 3 log scales** of the antimicrobial sample in comparison to the respective reference is used as assessment criterion to pass the antimicrobial test.

| Germ reduktion [log scales] | Antibacterial activity |
|---|---|
| < 3 | Not sufficient antimicrobial activity |
| ≥ 3 | Sufficient antimicrobial activity |

## References to Testconditions

| Testconditions | | |
|---|---|---|
| Sample size | 25 | $cm^2$ |
| Foil size | 16 | $cm^2$ |
| Volume Inoculum | 400 | µl |
| Sample cleaning | - | - |

## References to deviations, preincubations, special test conditions

NONE

| | |
|---|---|
| Work Order | 3250 |
| Setup-Code | 181218-10290-2801-01 |

## Test Results

| | Sample Name | Sample Code | $t_0$ (cells/cm$^2$) | | | $t_{24}$ (cells/cm$^2$) | | | Reduction [%] | Log Reduction |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Reference sample | 102902310180011 | 1.1 x $10^3$ | 8.3 x $10^2$ | 6.0 x $10^2$ | 2.3 x $10^2$ | 1.5 x $10^2$ | 1.5 x $10^2$ | | **Reference** |
| 2 | Liquid Guard 2,9% | 102902310180012 | | | | < 1.0 x $10^1$ | < 1.0 x $10^1$ | < 1.0 x $10^1$ | **> 99.99** | **3.45** |

*see “Interpretation of Results”, page 6

| | |
|---|---|
| **Test strain** | *Clostridioides difficile DSM 27543* |
| **Initial cell count inoculum / cm$^2$** | **1.25 x $10^4$** |
| **Initials of the editor** | MZ |
| **Measurement ended on** | Dec-12-2018 |

| | |
|---|---|
| Work Order | 3250 |
| Setup-Code | 181218-10290-2801-01 |

## Comments on test objects

NONE

## Interpretation of the results based on the measurements

Due to the test germ used *Clostridioides difficile* DSM 27543 exclusively grows anaerobic, under exclusion of air, the test specimens, and the agar plates were incubated after inoculation and incubation at 37 ° C in anaerobic pots. However, relatively low growth was seen on the blank immediately after inoculation (t0) and after incubation (t24). But there was a significant reduction in germs due to the Liquid Guard 2.9% coated Leneta film (102902310180012) compared to the blank sample (102902310180011).

**Editor:** Mr. Zehe ____ **Crosschecked:** Mr. Shendi ____

## References

JIS Z 2801:2012 Antimicrobial products – Test for antimicrobial activity and efficacy

| | |
|---|---|
| Work Order | 3223.3_Rev3 |
| Setup-Code | 190109-10290-2801-01 |

# Test Report

# JIS Z 2801:2012 (Mod)

## Antimicrobial products – Test for antimicrobial activity and efficacy

## Test Object:

*Coated leneta foil against Enterococcus hirae DSM3320 ATCC10541 after one year of artificial aging*

# Report on Findings

**Client:** Nano-Care UK (Signo-Nanocare UK Ltd)
**Address:** PO Box 225, Oswestry, Shropshire, SY10 1DL, UK +44 1691 654 282

**Work order no.:** 3223.3_Rev3

**Test object:** Coated leneta foil against *Enterococcus hirae* DSM3320 ATCC10541 after one year of artificial aging

**Sample description:** painted plastic films

**Date of receipt of sample:** 2018-Oct-22

**Type of test:** JIS Z 2801:2012 Antimicrobial products – Test for antimicrobial activity and efficacy

**Test Germ:** *Enterococcus hirae* DSM3320 ATCC10541

**Test laboratory:** QualityLabs BT GmbH

**Address:** Neumeyerstrasse 46a
90411 Nuremberg, Germany

**Setup-Code:** 190109-10290-2801-01

**Sample material:** Leneta foil

**No. of pages in report:** 7

**Report on findings to the client:**
**Place and date of preparation:** Nuremberg, 2019-Feb-27
**Recipient:** Nano-Care Deutschland GmbH
replaces the test report from the 20th Jan. 2019

**Laboratory Director:** _____
Harald Gerauer, Laboratory Director
QualityLabs BT GmbH

**Released:** _____
Markus Zehe, Managing Director
QualityLabs BT GmbH

## Declaration on Quality Assurance

This investigation was performed and supervised according to the standard operating procedure "SOP zu JIS Z 2801:2012 (Mod)" by QualityLabs BT GmbH. The laboratory and process are continually monitored by independent, external authorities, as well as by internal audits.

### Archiving

A copy of the test report, a protocol of the measurement as well as the accompanying correspondence and business records are archived by QualityLabs BT GmbH. The retention period is at least 10 years.

## Test description

Anti-bacterial activity is determined in accordance with a modified version of JIS Z 2801:2012.

During the test, a thin liquid-film containing the bacteria ($1.25 \times 10^4$ CFU / $cm^2$) is applied directly to the test sample (Standard: 5 cm x 5 cm). To avoid desiccation a foil (Standard: 4cm x 4cm, Stomacher Bags) is applied. Immediately after inoculation, the bacteria from the reference sample are separated from the sample and the enveloping foil surfaces using ultrasound and vortex devices and the number of viable germs (CFU – colony-forming units) is determined ($t_0$ value). A further set of reference samples and samples given anti-microbial treatment is incubated with bacteria in a liquid-film and the enveloping foil in a damp environment at 37°C. After 24 hours, the bacteria are separated from the sample surfaces using ultrasound and vortex devices and the number of viable germs is determined ($t_{24}$ value).

## Assessment of antimicrobial activity

A logarithmic germ reduction of **≥ 3 log scales** of the antimicrobial sample in comparison to the respective reference is used as assessment criterion to pass the antimicrobial test.

| Germ reduktion [log scales] | Antibacterial activity |
|---|---|
| < 3 | Not sufficient antimicrobial activity |
| ≥ 3 | Sufficient antimicrobial activity |

## References to Testconditions

| Test conditions | | |
|---|---|---|
| Sample size | 25 | $cm^2$ |
| Foil size | 16 | $cm^2$ |
| Volume Inoculum | 400 | µl |
| Sample cleaning | | - |

## References to deviations, preincubations, special test conditions

The samples have been stored for 56 days at 50 °C, which corresponds to an artificial aging of 1 year according to ASTM F 1980.

| | |
|---|---|
| Work Order | 3223.3_Rev3 |
| Setup-Code | 190109-10290-2801-01 |

# Test Results

| | Sample Name | Sample Code | $t_0$ (cells/cm$^2$) | | | $t_{24}$ (cells/cm$^2$) | | | Reduction [%] | Log Reduction |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Reference sample | 102902310180011 | 1.1 x $10^5$ | 9.4 x $10^4$ | 1.2 x $10^5$ | 1.3 x $10^5$ | 1.4 x $10^5$ | 7.6 x $10^4$ | | **Reference** |
| 2 | Liquid Guard 2,9% | 102902310180012 | | | | < 1.0 x $10^1$ | < 1.0 x $10^1$ | < 1.0 x $10^1$ | > 99.99 | **> 4** |

*see “Interpretation of Results”, page 6

| | |
|---|---|
| **Test strain** | *Enterococcus hirae DSM3320 ATCC10541* |
| **Initial cell count inoculum / cm$^2$** | **1.25 x $10^4$** |
| **Initials of the editor** | GM |
| **Measurement ended on** | 17-01-2019 |

| | |
|---|---|
| Work Order | 3223.3_Rev3 |
| Setup-Code | 190109-10290-2801-01 |

## Comments on test objects

NONE

## Interpretation of the results based on the measurements

NONE

**Editor:** Mr. Shendi____ **Crosschecked:** Mr. Mannala ____

## References

JIS Z 2801:2012 Antimicrobial products – Test for antimicrobial activity and efficacy

| | |
|---|---|
| Work Order | 3109.1 |
| Setup-Code | 180611-10290-2801-01 |

# Test Report

# JIS Z 2801:2012 (Mod)

## Antimicrobial products – Test for antimicrobial activity and efficacy

## Test Object:

*Coated Leneta-Foil vs. E. coli DSM 1576*

| | |
|---|---|
| Work Order | 3109.1 |
| Setup-Code | 180611-10290-2801-01 |

# Report on Findings

**Client:** Nano-Care UK (Signo-Nanocare UK Ltd)
**Address:** PO Box 225, Oswestry, Shropshire, SY10 1DL, UK +44 1691 654 282

**Work order no.:** 3109.1

**Test object:** Coated Leneta-Folie vs. E. coli DSM 1576

**Sample description:** lackierte Kunststofffolien

**Date of receipt of sample:** 08.06.2018

**Type of test:** JIS Z 2801:2012 Antimicrobial products – Test for antimicrobial activity and efficacy

**Test Germ:** Escherichia coli DSM1576 ATCC8739 ISML CC 02/023

**Test laboratory:** QualityLabs BT GmbH

**Address:** Neumeyerstrasse 46a
90411 Nuremberg, Germany

**Setup-Code:** 180611-10290-2801-01

**Sample material:** Leneta-Folie

**No. of pages in report:** 7

**Report on findings to the client:**
**Place and date of preparation:** Nuremberg, 14.9.2018
**Recipient:** Nano-Care Deutschland AG

**Laboratory Director:** _____
Harald Gerauer, Laboratory Director
QualityLabs BT GmbH

**Released:** _____
Markus Zehe, Managing Director
QualityLabs BT GmbH

## Declaration on Quality Assurance

This investigation was performed and supervised according to the standard operating procedure "SOP zu JIS Z 2801:2012 (Mod)" by QualityLabs BT GmbH. The laboratory and process are continually monitored by independent, external authorities, as well as by internal audits.

### Archiving

A copy of the test report, a protocol of the measurement as well as the accompanying correspondence and business records are archived by QualityLabs BT GmbH. The retention period is at least 10 years.

## Test description

Anti-bacterial activity is determined in accordance with a modified version of JIS Z 2801:2012.

During the test, a thin liquid-film containing the bacteria ($1.25 \times 10^4$ CFU / $cm^2$) is applied directly to the test sample (Standard: 5 cm x 5 cm). To avoid desiccation a foil (Standard: 4cm x 4cm, Stomacher Bags) is applied. Immediately after inoculation, the bacteria from the reference sample are separated from the sample and the enveloping foil surfaces using ultrasound and vortex devices and the number of viable germs (CFU – colony-forming units) is determined ($t_0$ value). A further set of reference samples and samples given anti-microbial treatment is incubated with bacteria in a liquid-film and the enveloping foil in a damp environment at 37°C. After 24 hours, the bacteria are separated from the sample surfaces using ultrasound and vortex devices and the number of viable germs is determined ($t_{24}$ value).

## Assessment of antimicrobial activity

A logarithmic germ reduction of **≥ 3 log scales** of the antimicrobial sample in comparison to the respective reference is used as assessment criterion to pass the antimicrobial test.

| Germ reduktion [log scales] | Antibacterial activity |
|---|---|
| < 3 | Not sufficient antimicrobial activity |
| ≥ 3 | Sufficient antimicrobial activity |

## References to Testconditions

| Testconditions | | |
|---|---|---|
| Sample size | 25 | $cm^2$ |
| Foil size | 16 | $cm^2$ |
| Volume Inoculum | 400 | µl |
| Sample cleaning | Isopropanol | - |

## References to deviations, preincubations, special test conditions

NONE

| | |
|---|---|
| Work Order | 3109.1 |
| Setup-Code | 180611-10290-2801-01 |

## Test Results

| | Sample Name | Sample Code | $t_0$ (cells/cm$^2$) | | | $t_{24}$ (cells/cm$^2$) | | | Reduction [%] | Log Reduction |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Leneta-Folie P121-10 (Reference) | 102900806180001 | 9.0 x $10^4$ | 1.1 x $10^5$ | 1.1 x $10^5$ | 5.1 x $10^5$ | 4.1 x $10^5$ | 5.3 x $10^5$ | - | - |
| 2 | Liquid Guard clean + primer + wipe | 102900806180002 | | | | < 1.0 x $10^1$ | < 1.0 x $10^1$ | < 1.0 x $10^1$ | **> 99.99** | **> 4** |
| 3 | Liquid Guard pro + wipe | 102900806180003 | | | | < 1.0 x $10^1$ | < 1.0 x $10^1$ | < 1.0 x $10^1$ | **> 99.99** | **> 4** |

*see “Interpretation of Results”, page 6

| | |
|---|---|
| **Test strain** | *Escherichia coli DSM1576 ATCC8739 ISML CC 02/023* |
| **Initial cell count inoculum / cm$^2$** | **1.25 x $10^4$** |
| **Initials of the editor** | MZ |
| **Measurement ended on** | Jun-15-2018 |

| | |
|---|---|
| Work Order | 3109.1 |
| Setup-Code | 180611-10290-2801-01 |

## Comments on test objects

NONE

## Interpretation of the results based on the measurements

NONE

**Editor:** Mr. Zehe ____ **Crosschecked:** Mr. Shendi ____

## References

JIS Z 2801:2012 Antimicrobial products – Test for antimicrobial activity and efficacy

| | |
|---|---|
| Work Order | 3382 |
| Setup-Code | 190710-10290-2801-01 |

# Test Report

# JIS Z 2801:2012 (Mod)

## Antimicrobial products – Test for antimicrobial activity and efficacy

## Test Object:

*Liquid Guard 2,9 % versus Escherichia coli DSM22312*

| | |
|---|---|
| Work Order | 3382 |
| Setup-Code | 190710-10290-2801-01 |

# Report on Findings

**Client:** Nano-Care UK (Signo-Nanocare UK Ltd)
**Address:** PO Box 225, Oswestry, Shropshire, SY10 1DL, UK +44 1691 654 282

**Work order no.:** 3382

**Test object:** Liquid Guard 2,9 % versus *Escherichia coli* DSM22312

**Sample description:** Coated foil

**Date of receipt of sample:** 2019-Jul-03

**Type of test:** JIS Z 2801:2012 Antimicrobial products – Test for antimicrobial activity and efficacy

**Test Germ:** *Escherichia coli* DSM22312

**Test laboratory:** QualityLabs BT GmbH

**Address:** Neumeyerstrasse 46a
90411 Nuremberg, Germany

**Setup-Code:** 190710-10290-2801-01

**Sample material:** n.b.

**No. of pages in report:** 7

**Report on findings to the client:**
**Place and date of preparation:** Nuremberg, 2019-Jul-18
**Recipient:** Nano-Care Deutschland GmbH

**Laboratory Director:**

_____
Harald Gerauer, Laboratory Director
QualityLabs BT GmbH

**Released:**

_____
Markus Zehe, Managing Director
QualityLabs BT GmbH

| | |
|---|---|
| Work Order | 3382 |
| Setup-Code | 190710-10290-2801-01 |

## Declaration on Quality Assurance

This investigation was performed and supervised according to the standard operating procedure "SOP zu JIS Z 2801:2012 (Mod)" by QualityLabs BT GmbH. The laboratory and process are continually monitored by independent, external authorities, as well as by internal audits.

### Archiving

A copy of the test report, a protocol of the measurement as well as the accompanying correspondence and business records are archived by QualityLabs BT GmbH. The retention period is at least 10 years.

## Test description

Anti-bacterial activity is determined in accordance with a modified version of JIS Z 2801:2012.

During the test, a thin liquid-film containing the bacteria ($1.25 \times 10^4$ CFU / $cm^2$) is applied directly to the test sample (Standard: 5 cm x 5 cm). To avoid desiccation a foil (Standard: 4cm x 4cm, Stomacher Bags) is applied. Immediately after inoculation, the bacteria from the reference sample are separated from the sample and the enveloping foil surfaces using ultrasound and vortex devices and the number of viable germs (CFU – colony-forming units) is determined ($t_0$ value). A further set of reference samples and samples given anti-microbial treatment is incubated with bacteria in a liquid-film and the enveloping foil in a damp environment at 37°C. After 24 hours, the bacteria are separated from the sample surfaces using ultrasound and vortex devices and the number of viable germs is determined ($t_{24}$ value).

## Assessment of antimicrobial activity

A logarithmic germ reduction of **≥ 3 log scales** of the antimicrobial sample in comparison to the respective reference is used as assessment criterion to pass the antimicrobial test.

| Germ reduktion [log scales] | Antibacterial activity |
|---|---|
| < 3 | Not sufficient antimicrobial activity |
| ≥ 3 | Sufficient antimicrobial activity |

## References to Testconditions

| Testconditions | | |
| --- | --- | --- |
| Sample size | 25 | $cm^2$ |
| Foil size | 16 | $cm^2$ |
| Volume Inoculum | 400 | µl |
| Sample cleaning | | - |

## References to deviations, preincubations, special test conditions

NONE

| | |
|---|---|
| Work Order | 3382 |
| Setup-Code | 190710-10290-2801-01 |

## Test Results

| | Sample Name | Sample Code | $t_0$ (cells/cm$^2$) | | | $t_{24}$ (cells/cm$^2$) | | | Reduction [%] | Log Reduction |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Reference sample | 102901007190019 | 7.7 x $10^4$ | 10.0 x $10^4$ | 8.9 x $10^4$ | 8.6 x $10^4$ | 9.9 x $10^4$ | 1.1 x $10^5$ | | **Reference** |
| 2 | Liquid Guard 2,9% | 102901007190020 | | | | < 1.0 x $10^1$ | < 1.0 x $10^1$ | < 1.0 x $10^1$ | > 99.99 | **> 4** |

*see “Interpretation of Results”, page 6

| | |
|---|---|
| **Test strain** | *Escherichia coli DSM22312* |
| **Initial cell count inoculum / cm$^2$** | **1.25 x $10^4$** |
| **Initials of the editor** | MZ |
| **Measurement ended on** | Jul-12-2019 |

## Comments on test objects

NONE

## Interpretation of the results based on the measurements

NONE

**Editor:** Mr. Zehe ____ **Crosschecked:** Mr. Mannala ____

## References

JIS Z 2801:2012 Antimicrobial products – Test for antimicrobial activity and efficacy

| | |
|---|---|
| Work Order | 3109.3 |
| Setup-Code | 180611-10290-2801-03 |

# Test Report

# JIS Z 2801:2012 (Mod)

## Antimicrobial products – Test for antimicrobial activity and efficacy

## Test Object:

*Coated Leneta Foil vs. Klebsiella pneumoniae DSM 6135*

# Report on Findings

**Client:** Nano-Care UK (Signo-Nanocare UK Ltd)
**Address:** PO Box 225, Oswestry, Shropshire, SY10 1DL, UK +44 1691 654 282

**Work order no.:** 3109.3

**Test object:** Coated Leneta Foil vs. Klebsiella pneumoniae DSM 6135

**Sample description:** Coated Foil

**Date of receipt of sample:** 08.06.2018

**Type of test:** JIS Z 2801:2012 Antimicrobial products – Test for antimicrobial activity and efficacy

**Test Germ:** Klebsiella pneumoniae DSM6135

**Test laboratory:** QualityLabs BT GmbH

**Address:** Neumeyerstrasse 46a
90411 Nuremberg, Germany

**Setup-Code:** 180611-10290-2801-03

**Sample material:** Leneta Foil

**No. of pages in report:** 7

**Report on findings to the client:**
**Place and date of preparation:** Nuremberg, 14.9.2018
**Recipient:** Nano-Care Deutschland AG

**Laboratory Director:** _____
Harald Gerauer, Laboratory Director
QualityLabs BT GmbH

**Released:** _____
Markus Zehe, Managing Director
QualityLabs BT GmbH

| | |
|---|---|
| Work Order | 3109.3 |
| Setup-Code | 180611-10290-2801-03 |

## Declaration on Quality Assurance

This investigation was performed and supervised according to the standard operating procedure "SOP zu JIS Z 2801:2012 (Mod)" by QualityLabs BT GmbH. The laboratory and process are continually monitored by independent, external authorities, as well as by internal audits.

### Archiving

A copy of the test report, a protocol of the measurement as well as the accompanying correspondence and business records are archived by QualityLabs BT GmbH. The retention period is at least 10 years.

## Test description

Anti-bacterial activity is determined in accordance with a modified version of JIS Z 2801:2012.

During the test, a thin liquid-film containing the bacteria ($1.25 \times 10^4$ CFU / $cm^2$) is applied directly to the test sample (Standard: 5 cm x 5 cm). To avoid desiccation a foil (Standard: 4cm x 4cm, Stomacher Bags) is applied. Immediately after inoculation, the bacteria from the reference sample are separated from the sample and the enveloping foil surfaces using ultrasound and vortex devices and the number of viable germs (CFU – colony-forming units) is determined ($t_0$ value). A further set of reference samples and samples given anti-microbial treatment is incubated with bacteria in a liquid-film and the enveloping foil in a damp environment at 37°C. After 24 hours, the bacteria are separated from the sample surfaces using ultrasound and vortex devices and the number of viable germs is determined ($t_{24}$ value).

## Assessment of antimicrobial activity

A logarithmic germ reduction of **≥ 3 log scales** of the antimicrobial sample in comparison to the respective reference is used as assessment criterion to pass the antimicrobial test.

| Germ reduktion [log scales] | Antibacterial activity |
|---|---|
| < 3 | Not sufficient antimicrobial activity |
| ≥ 3 | Sufficient antimicrobial activity |

| | |
|---|---|
| Work Order | 3109.3 |
| Setup-Code | 180611-10290-2801-03 |

## References to Testconditions

| Testconditions | | |
|---|---|---|
| Sample size | 25 | $cm^2$ |
| Foil size | 16 | $cm^2$ |
| Volume Inoculum | 400 | µl |
| Sample cleaning | Isopropanol | - |

## References to deviations, preincubations, special test conditions

NONE

| | |
|---|---|
| Work Order | 3109.3 |
| Setup-Code | 180611-10290-2801-03 |

## Test Results

| | Sample Name | Sample Code | $t_0$ (cells/cm$^2$) | | | $t_{24}$ (cells/cm$^2$) | | | Reduction [%] | Log Reduction |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Leneta-Folie P121-10 (Reference) | 102900806180001 | 2.1 x $10^4$ | 3.6 x $10^4$ | 8.0 x $10^4$ | 2.7 x $10^5$ | 3.8 x $10^5$ | 2.7 x $10^5$ | - | - |
| 2 | Liquid Guard clean + primer + wipe | 102900806180002 | | | | 6.4 x $10^2$ | 7.5 x $10^1$ | < 1.0 x $10^1$ | **99.92** | **3.11** |
| 3 | Liquid Guard pro + wipe | 102900806180003 | | | | < 1.0 x $10^1$ | < 1.0 x $10^1$ | < 1.0 x $10^1$ | **> 99.99** | **> 4** |

*see “Interpretation of Results”, page 6

| | |
|---|---|
| **Test strain** | *Klebsiella pneumoniae DSM6135* |
| **Initial cell count inoculum / cm$^2$** | **1.25 x $10^4$** |
| **Initials of the editor** | MZ |
| **Measurement ended on** | Mar-12-20xx |

| | |
|---|---|
| Work Order | 3109.3 |
| Setup-Code | 180611-10290-2801-03 |

## Comments on test objects

NONE

## Interpretation of the results based on the measurements

NONE

**Editor:** Mr. Zehe ____ **Crosschecked:** Mr. Shendi ____

## References

JIS Z 2801:2012 Antimicrobial products – Test for antimicrobial activity and efficacy

| | |
|---|---|
| Work Order | 3109.6 |
| Setup-Code | 180612-10290-2801-03 |

# Test Report

# JIS Z 2801:2012 (Mod)

## Antimicrobial products – Test for antimicrobial activity and efficacy

## Test Object:

## Coated Leneta Foil vs. Listeria monocytogenes DSM 15675

# Report on Findings

**Client:**
**Address:** Nano-Care UK (Signo-Nanocare UK Ltd)
PO Box 225, Oswestry, Shropshire, SY10 1DL, UK +44 1691 654 282

**Work order no.:** 3109.6

**Test object:** Coated Leneta Foil vs. Listeria monocytogenes DSM 15675

**Sample description:** Coated Leneta Foil

**Date of receipt of sample:** 14.09.2018

**Type of test:** JIS Z 2801:2012 Antimicrobial products – Test for antimicrobial activity and Efficacy

**Test Germ:** Listeria monocytogenes DSM15675

**Test laboratory:** QualityLabs BT GmbH

**Address:** Neumeyerstrasse 46a
90411 Nuremberg, Germany

**Setup-Code:** 180612-10290-2801-03

**Sample material:** Leneta Foil

**No. of pages in report:** 7

**Report on findings to the client:**
**Place and date of preparation:** Nuremberg, 14.9.2018
**Recipient:** Nano-Care Deutschland AG

**Laboratory Director:** _____
Harald Gerauer, Laboratory Director
QualityLabs BT GmbH

**Released:** _____
Markus Zehe, Managing Director
QualityLabs BT GmbH

| | |
|---|---|
| Work Order | 3109.6 |
| Setup-Code | 180612-10290-2801-03 |

## Declaration on Quality Assurance

This investigation was performed and supervised according to the standard operating procedure “SOP zu JIS Z 2801:2012 (Mod)" by QualityLabs BT GmbH. The laboratory and process are continually monitored by independent, external authorities, as well as by internal audits.

### Archiving

A copy of the test report, a protocol of the measurement as well as the accompanying correspondence and business records are archived by QualityLabs BT GmbH. The retention period is at least 10 years.

## Test description

Anti-bacterial activity is determined in accordance with a modified version of JIS Z 2801:2012.

During the test, a thin liquid-film containing the bacteria (1.25 x $10^4$ CFU / $cm^2$) is applied directly to the test sample (Standard: 5 cm x 5 cm). To avoid desiccation a foil (Standard: 4cm x 4cm, Stomacher Bags) is applied. Immediately after inoculation, the bacteria from the reference sample are separated from the sample and the enveloping foil surfaces using ultrasound and vortex devices and the number of viable germs (CFU – colony-forming units) is determined ($t_0$ value). A further set of reference samples and samples given anti-microbial treatment is incubated with bacteria in a liquid-film and the enveloping foil in a damp environment at 37°C. After 24 hours, the bacteria are separated from the sample surfaces using ultrasound and vortex devices and the number of viable germs is determined ($t_{24}$ value).

## Assessment of antimicrobial activity

A logarithmic germ reduction of **≥ 3 log scales** of the antimicrobial sample in comparison to the respective reference is used as assessment criterion to pass the antimicrobial test.

| Germ reduktion [log scales] | Antibacterial activity |
|---|---|
| < 3 | Not sufficient antimicrobial activity |
| ≥ 3 | Sufficient antimicrobial activity |

| | |
|---|---|
| Work Order | 3109.6 |
| Setup-Code | 180612-10290-2801-03 |

## References to Testconditions

| Testconditions | | |
|---|---|---|
| Sample size | 25 | $cm^2$ |
| Foil size | 16 | $cm^2$ |
| Volume Inoculum | 400 | µl |
| Sample cleaning | Isopropanol | - |

## References to deviations, preincubations, special test conditions

NONE

| | |
|---|---|
| Work Order | 3109.6 |
| Setup-Code | 180612-10290-2801-03 |

## Test Results

| | Sample Name | Sample Code | $t_0$ (cells/cm$^2$) | | | $t_{24}$ (cells/cm$^2$) | | | Reduction [%] | Log Reduction |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Leneta-Folie P121-10 (Reference) | 102900806180001 | 1,3 x $10^5$ | 1,1 x $10^5$ | 1,4 x $10^5$ | 1,7 x $10^4$ | 2,9 x $10^4$ | 2,0 x $10^5$ | - | - |
| 2 | Liquid Guard clean + primer + wipe | 102900806180002 | | | | < 1,0 x $10^1$ | < 1,0 x $10^1$ | < 1,0 x $10^1$ | **> 99,99** | **> 4** |
| 3 | Liquid Guard pro +wipe | 102900806180003 | | | | < 1,0 x $10^1$ | < 1,0 x $10^1$ | < 1,0 x $10^1$ | **> 99,99** | **> 4** |

*see “Interpretation of Results”, page 6

| | |
|---|---|
| **Test strain** | *Listeria monocytogenes DSM15675* |
| **Initial cell count inoculum / cm$^2$** | **1.25 x $10^4$** |
| **Initials of the editor** | OS |
| **Measurement ended on** | 18.06.2018 |

| | |
|---|---|
| Work Order | 3109.6 |
| Setup-Code | 180612-10290-2801-03 |

## Comments on test objects

NONE

## Interpretation of the results based on the measurements

NONE

**Editor:** Mr. Shendi ____ **Crosschecked:** Mr.Zehe ____

## References

JIS Z 2801:2012 Antimicrobial products – Test for antimicrobial activity and efficacy

| | |
|---|---|
| Work Order | 3212.1 |
| Setup-Code | 181029-10290-2801-01 |

# Test Report

# JIS Z 2801:2012 (Mod)

## Antimicrobial products – Test for antimicrobial activity and efficacy

## Test Object:

*Coated Leneta-Foil vs. Pseudomonas aeruginosa EDCC 5272*

# Report on Findings

**Client:** Nano-Care UK (Signo-Nanocare UK Ltd)
**Address:** PO Box 225, Oswestry, Shropshire, SY10 1DL, UK +44 1691 654 282

**Work order no.:** 3212.1

**Test object:** Coated Leneta-Foil vs. *Pseudomonas aeruginosa* EDCC 5272

**Sample description:** coated foil

**Date of receipt of sample:** 2018-Oct-22

**Type of test:** JIS Z 2801:2012 Antimicrobial products – Test for antimicrobial activity and efficacy

**Test Germ:** *Pseudomonas aeruginosa* EDCC 5272

**Test laboratory:** QualityLabs BT GmbH

**Address:** Neumeyerstrasse 46a
90411 Nuremberg, Germany

**Setup-Code:** 181029-10290-2801-01

**Sample material:** n.b.

**No. of pages in report:** 7

**Report on findings to the client:** **Place and date of preparation:** Nuremberg, 2018-Nov-07
**Recipient:** Nano-Care Deutschland GmbH

**Laboratory Director:** _____
Harald Gerauer, Laboratory Director
QualityLabs BT GmbH

**Released:** _____
Markus Zehe, Managing Director
QualityLabs BT GmbH

## Declaration on Quality Assurance

This investigation was performed and supervised according to the standard operating procedure "SOP zu JIS Z 2801:2012 (Mod)" by QualityLabs BT GmbH. The laboratory and process are continually monitored by independent, external authorities, as well as by internal audits.

### Archiving

A copy of the test report, a protocol of the measurement as well as the accompanying correspondence and business records are archived by QualityLabs BT GmbH. The retention period is at least 10 years.

## Test description

Anti-bacterial activity is determined in accordance with a modified version of JIS Z 2801:2012.

During the test, a thin liquid-film containing the bacteria ($1.25 \times 10^4$ CFU / $cm^2$) is applied directly to the test sample (Standard: 5 cm x 5 cm). To avoid desiccation a foil (Standard: 4cm x 4cm, Stomacher Bags) is applied. Immediately after inoculation, the bacteria from the reference sample are separated from the sample and the enveloping foil surfaces using ultrasound and vortex devices and the number of viable germs (CFU – colony-forming units) is determined ($t_0$ value). A further set of reference samples and samples given anti-microbial treatment is incubated with bacteria in a liquid-film and the enveloping foil in a damp environment at 37°C. After 24 hours, the bacteria are separated from the sample surfaces using ultrasound and vortex devices and the number of viable germs is determined ($t_{24}$ value).

## Assessment of antimicrobial activity

A logarithmic germ reduction of **≥ 3 log scales** of the antimicrobial sample in comparison to the respective reference is used as assessment criterion to pass the antimicrobial test.

| Germ reduktion [log scales] | Antibacterial activity |
|---|---|
| < 3 | Not sufficient antimicrobial activity |
| ≥ 3 | Sufficient antimicrobial activity |

| | |
|---|---|
| Work Order | 3212.1 |
| Setup-Code | 181029-10290-2801-01 |

## References to Testconditions

| Testconditions | | |
|---|---|---|
| Sample size | 25 | $cm^2$ |
| Foil size | 16 | $cm^2$ |
| Volume Inoculum | 300 | µl |
| Sample cleaning | | - |

## References to deviations, preincubations, special test conditions

NONE

| | |
|---|---|
| Work Order | 3212.1 |
| Setup-Code | 181029-10290-2801-01 |

## Test Results

| | Sample Name | Sample Code | $t_0$ (cells/cm$^2$) | | | $t_{24}$ (cells/cm$^2$) | | | Reduction [%] | Log Reduction |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Reference | 102902310180011 | 5.3 x $10^4$ | 4.9 x $10^4$ | 4.2 x $10^4$ | 4.1 x $10^5$ | 3.5 x $10^5$ | 4.7 x $10^5$ | | **Reference** |
| 2 | Liquid Guard 2,9% | 102902310180012 | | | | < 1.0 x $10^1$ | < 1.0 x $10^1$ | < 1.0 x $10^1$ | **> 99.99** | **> 4** |

*see “Interpretation of Results”, page 6

| | |
|---|---|
| **Test strain** | *Pseudomonas aeruginosa EDCC 5272* |
| **Initial cell count inoculum / cm$^2$** | **1.25 x $10^4$** |
| **Initials of the editor** | MZ |
| **Measurement ended on** | Nov-11-2018 |

| | |
|---|---|
| Work Order | 3212.1 |
| Setup-Code | 181029-10290-2801-01 |

## Comments on test objects

NONE

## Interpretation of the results based on the measurements

NONE

**Editor:** Mr. Zehe ____ **Crosschecked:** Mr. Mannala ____

## References

JIS Z 2801:2012 Antimicrobial products – Test for antimicrobial activity and efficacy

| | |
|---|---|
| Work Order | 3109.5 |
| Setup-Code | 180612-10290-2801-02 |

# Test Report

# JIS Z 2801:2012 (Mod)

## Antimicrobial products – Test for antimicrobial activity and Effectiveness

## Test Object:

*Coated Leneta - Foil vs. Salmonella choleraesuis DSM11320*

# Report on Findings

**Client:** Nano-Care UK (Signo-Nanocare UK Ltd)
**Address:** PO Box 225, Oswestry, Shropshire, SY10 1DL, UK +44 1691 654 282

**Work order no.:** 3109.5

**Test object:** Coated Leneta Foil vs. Salmonella choleraesuis DSM11320

**Sample description:** Coated Leneta Foil

**Date of receipt of sample:** 14.09.2018

**Type of test:** JIS Z 2801:2012 Antimicrobial products – Test for antimicrobial activity and Efficacy

**Test Germ:** Salmonella choleraesuis DSM11320

**Test laboratory:** QualityLabs BT GmbH

**Address:** Neumeyerstrasse 46a
90411 Nuremberg, Germany

**Setup-Code:** 180612-10290-2801-02

**Sample material:** Leneta Foil

**No. of pages in report:** 7

**Report on findings to the client:**
**Place and date of preparation:** Nuremberg, 14.9.2018
**Recipient:** Nano-Care Deutschland AG

**Laboratory Director:** _____
Harald Gerauer, Laboratory Director
QualityLabs BT GmbH

**Released:** _____
Markus Zehe, Managing Director
QualityLabs BT GmbH

## Declaration on Quality Assurance

This investigation was performed and supervised according to the standard operating procedure "SOP zu JIS Z 2801:2012 (Mod)" by QualityLabs BT GmbH. The laboratory and process are continually monitored by independent, external authorities, as well as by internal audits.

### Archiving

A copy of the test report, a protocol of the measurement as well as the accompanying correspondence and business records are archived by QualityLabs BT GmbH. The retention period is at least 10 years.

## Test description

Anti-bacterial activity is determined in accordance with a modified version of JIS Z 2801:2012.

During the test, a thin liquid-film containing the bacteria (1.25 x $10^4$ CFU / $cm^2$) is applied directly to the test sample (Standard: 5 cm x 5 cm). To avoid desiccation a foil (Standard: 4cm x 4cm, Stomacher Bags) is applied. Immediately after inoculation, the bacteria from the reference sample are separated from the sample and the enveloping foil surfaces using ultrasound and vortex devices and the number of viable germs (CFU – colony-forming units) is determined ($t_0$ value). A further set of reference samples and samples given anti-microbial treatment is incubated with bacteria in a liquid-film and the enveloping foil in a damp environment at 37°C. After 24 hours, the bacteria are separated from the sample surfaces using ultrasound and vortex devices and the number of viable germs is determined ($t_{24}$ value).

## Assessment of antimicrobial activity

A logarithmic germ reduction of **≥ 3 log scales** of the antimicrobial sample in comparison to the respective reference is used as assessment criterion to pass the antimicrobial test.

| Germ reduktion [log scales] | Antibacterial activity |
|---|---|
| < 3 | Not sufficient antimicrobial activity |
| ≥ 3 | Sufficient antimicrobial activity |

| | |
|---|---|
| Work Order | 3109.5 |
| Setup-Code | 180612-10290-2801-02 |

## References to Testconditions

| Testconditions | | |
|---|---|---|
| Sample size | 25 | $cm^2$ |
| Foil size | 16 | $cm^2$ |
| Volume Inoculum | 400 | µl |
| Sample cleaning | Isopropanol | - |

## References to deviations, preincubations, special test conditions

NONE

| | |
|---|---|
| Work Order | 3109.5 |
| Setup-Code | 180612-10290-2801-02 |

# Test Results

| | Sample Name | Sample Code | $t_0$ (cells/cm$^2$) | | | $t_{24}$ (cells/cm$^2$) | | | Reduction [%] | Log Reduction |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Leneta-Foil P121-10 (Reference) | 102900806180001 | 6,6 x $10^4$ | 5,5 x $10^4$ | 7,1 x $10^4$ | 7,8 x $10^4$ | 7,3 x $10^4$ | 1,2 x $10^5$ | - | - |
| 2 | Liquid Guard clean + primer + wipe | 102900806180002 | | | | < 1,0 x $10^1$ | < 1,0 x $10^1$ | < 1,0 x $10^1$ | **> 99,99** | **> 4** |
| 3 | Liquid Guard pro + wipe | 102900806180003 | | | | < 1,0 x $10^1$ | < 1,0 x $10^1$ | < 1,0 x $10^1$ | **> 99,99** | **> 4** |

*see “Interpretation of Results”, page 6

| | |
|---|---|
| **Test strain** | *Salmonella choleraesuis DSM11320* |
| **Initial cell count inoculum / cm$^2$** | **1.25 x $10^4$** |
| **Initials of the editor** | OS |
| **Measurement ended on** | 15.06.2018 |

| | |
|---|---|
| Work Order | 3109.5 |
| Setup-Code | 180612-10290-2801-02 |

## Comments on test objects

NONE

## Interpretation of the results based on the measurements

NONE

**Editor:** Mr. Shendi ____ **Crosschecked:** Mr. Zehe____

## References

JIS Z 2801:2012 Antimicrobial products – Test for antimicrobial activity and Efficacy

| | |
|---|---|
| Work Order | 3109.2 |
| Setup-Code | 180611-10290-2801-02 |

# Test Report

# JIS Z 2801:2012 (Mod)

## Antimicrobial products – Test for antimicrobial activity and efficacy

## Test Object:

*Coated Leneta-Foil vs. Staph. aureus DSM 21979*

| | |
|---|---|
| Work Order | 3109.2 |
| Setup-Code | 180611-10290-2801-02 |

# Report on Findings

**Client:** Nano-Care UK (Signo-Nanocare UK Ltd)
**Address:** PO Box 225, Oswestry, Shropshire, SY10 1DL, UK +44 1691 654 282

**Work order no.:** 3109.2

**Test object:** Coated Leneta-Foil vs. Staph. aureus DSM 21979

**Sample description:** Coated Foil

**Date of receipt of sample:** 08.06.2018

**Type of test:** JIS Z 2801:2012 Antimicrobial products – Test for antimicrobial activity and efficacy

**Test Germ:** Staphylococcus aureus DSM21979 EDCC 5247

**Test laboratory:** QualityLabs BT GmbH

**Address:** Neumeyerstrasse 46a
90411 Nuremberg, Germany

**Setup-Code:** 180611-10290-2801-02

**Sample material:** Leneta Foil

**No. of pages in report:** 7

**Report on findings to the client:**
**Place and date of preparation:** Nuremberg, 13.9.2018
**Recipient:** Nano-Care Deutschland AG

**Laboratory Director:** _____
Harald Gerauer, Laboratory Director
QualityLabs BT GmbH

**Released:** _____
Markus Zehe, Managing Director
QualityLabs BT GmbH

## Declaration on Quality Assurance

This investigation was performed and supervised according to the standard operating procedure "SOP zu JIS Z 2801:2012 (Mod)" by QualityLabs BT GmbH. The laboratory and process are continually monitored by independent, external authorities, as well as by internal audits.

### Archiving

A copy of the test report, a protocol of the measurement as well as the accompanying correspondence and business records are archived by QualityLabs BT GmbH. The retention period is at least 10 years.

## Test description

Anti-bacterial activity is determined in accordance with a modified version of JIS Z 2801:2012.

During the test, a thin liquid-film containing the bacteria ($1.25 \times 10^4$ CFU / $cm^2$) is applied directly to the test sample (Standard: 5 cm x 5 cm). To avoid desiccation a foil (Standard: 4cm x 4cm, Stomacher Bags) is applied. Immediately after inoculation, the bacteria from the reference sample are separated from the sample and the enveloping foil surfaces using ultrasound and vortex devices and the number of viable germs (CFU – colony-forming units) is determined ($t_0$ value). A further set of reference samples and samples given anti-microbial treatment is incubated with bacteria in a liquid-film and the enveloping foil in a damp environment at 37°C. After 24 hours, the bacteria are separated from the sample surfaces using ultrasound and vortex devices and the number of viable germs is determined ($t_{24}$ value).

## Assessment of antimicrobial activity

A logarithmic germ reduction of **≥ 3 log scales** of the antimicrobial sample in comparison to the respective reference is used as assessment criterion to pass the antimicrobial test.

| Germ reduktion [log scales] | Antibacterial activity |
|---|---|
| < 3 | Not sufficient antimicrobial activity |
| ≥ 3 | Sufficient antimicrobial activity |

| | |
|---|---|
| Work Order | 3109.2 |
| Setup-Code | 180611-10290-2801-02 |

## References to Testconditions

| Testconditions | | |
|---|---|---|
| Sample size | 25 | $cm^2$ |
| Foil size | 16 | $cm^2$ |
| Volume Inoculum | 400 | µl |
| Sample cleaning | Isopropanol | - |

## References to deviations, preincubations, special test conditions

NONE

| | |
|---|---|
| Work Order | 3109.2 |
| Setup-Code | 180611-10290-2801-02 |

## Test Results

| | Sample Name | Sample Code | $t_0$ (cells/cm$^2$) | | | $t_{24}$ (cells/cm$^2$) | | | Reduction [%] | Log Reduction |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Leneta-Folie P121-10 (Reference) | 102900806180001 | 9.0 x $10^4$ | 1.1 x $10^5$ | 1.1 x $10^5$ | 5.1 x $10^5$ | 4.1 x $10^5$ | 5.3 x $10^5$ | - | - |
| 2 | Liquid Guard clean + primer + wipe | 102900806180002 | | | | < 1.0 x $10^1$ | < 1.0 x $10^1$ | < 1.0 x $10^1$ | **> 99.99** | **> 4** |
| 3 | Liquid Guard pro + wipe | 102900806180003 | | | | < 1.0 x $10^1$ | < 1.0 x $10^1$ | < 1.0 x $10^1$ | **> 99.99** | **> 4** |

*see “Interpretation of Results”, page 6

| | |
|---|---|
| **Test strain** | *Staphylococcus aureus DSM21979 EDCC 5247* |
| **Initial cell count inoculum / cm$^2$** | **1.25 x $10^4$** |
| **Initials of the editor** | MZ |
| **Measurement ended on** | Mar-12-20xx |

| | |
|---|---|
| Work Order | 3109.2 |
| Setup-Code | 180611-10290-2801-02 |

## Comments on test objects

NONE

## Interpretation of the results based on the measurements

NONE

**Editor:** Mr. Zehe ____ **Crosschecked:** Mr. Shendi ____

## References

JIS Z 2801:2012 Antimicrobial products – Test for antimicrobial activity and efficacy