



Microbiology
Location Zeist
Utrechtseweg 48
P.O. Box 360
3700 AJ Zeist
The Netherlands

TNO report

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Antibacterial activity of Marmoleum Topshield
according to the TNO Seedlayer method

www.tno.nl

T +31 30 694 41 44
F +31 30 695 72 24
infofood@voeding.tno.nl

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|----------------------|---------------------|
| Date | August 2, 2004 |
| Author | B.J. Hartog, M.Sc. |
| Technician | Mrs. H.C.M. Vissers |
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1 Introduction

Forbo Linoleum B.V., Krommenie (NL) developed the new linoleum flooring type Marmoleum Topshield.

By order of Forbo Linoleum B.V. (Forbo ordernumber 4500034258) the antibacterial activity of Marmoleum Topshield has been tested according to the TNO Seedlayer method, using the bacterial strains *Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus* RIVM 89-646 (MRSA strain) and *Listeria monocytogenes* ATCC 19114 as the test organisms, by TNO Nutrition and Food Research, Zeist (NL). For this purpose Forbo Linoleum B.V. provided a sample of Marmoleum Topshield as the test specimen. Pieces of the test specimen have been tested as such and after leaching by soaking with water.

The results of the tests are presented in this report.

2 Materials and methods

2.1 Test specimens

A sample of Marmoleum Topshield was provided by Forbo Linoleum B.V., Krommenie (NL) as the test specimen.

The sample was received on 21 June 2004 at the TNO test facilities in Zeist and registered under the code 3119/04/0380.

A sample of polystyrene without antimicrobial activity, provided by TNO, was used as negative control specimen.

Preparation of the test specimens

Before testing, the surface of the test specimens was cleaned by wiping with ethanol and air dried. So treated pieces of the test specimen have been tested as such, and after leaching by soaking in an excess of water for 1 hour at ambient temperature and air-drying afterwards.

2.2 TNO Seedlayer Method

2.2.1 *Principle*

The Seedlayer Method is an agar diffusion type method, intended to determine qualitatively the antimicrobial activity of materials. Small teflon rings, containing a solid culture medium inoculated with a test organism (the seedlayer), are positioned on the material under test. After 1, 2, 3 and 4 days of incubation the seedlayer is examined for growth inhibition of the test organisms.

2.2.2 *Test organisms*

The following bacterial strains were used as the test organisms:

1. *Staphylococcus aureus* ATCC 6538;
2. *Staphylococcus aureus* RIVM 89-646 (MRSA strain);
3. *Listeria monocytogenes* ATCC19114 (bacterium);

In table 1 the main characteristics of the organisms are summarized.

2.2.3 *Inoculum and seedlayer preparation*

The strains of the different testorganisms were maintained according to the TNO standard operational procedure for the maintenance of culture collection strains.

Fresh bacterial cells were cultivated on Trypticase Soy Agar (TSA) slants for 24 hours at 30°C. The bacterial cells were harvested by washing off the surface of the solid culture media using sterile physiological salt solution (PS). The obtained cell suspensions were further diluted to adjust the required level in the seedlayer test.

To prepare seedlayers for each test organism the prescribed medium (see 2.2.4) was inoculated with the diluted cell suspension to the level of 10^5 cfu (colonyforming units)/ml. To facilitate observation of (micro)-colonies of the test organism growing in the seedlayer, the following growth-indicators were added to the seedlayer media:

- 1% TTC (Triphenyl Tetrazolium Chloride, Merck 8380) for the bacterial test strains 1 and 2;
- 1% tellurite (potassium tellurite, Merck 5164) for the bacterial strain 3.

2.2.4 *Seedlayer media*

For the bacterial testorganisms Antibiotic Agar no.1 (Oxoid CM 327), pH 7.0 was used as the seedlayer medium.

2.2.5 *Test procedure*

For each of the test organisms teflon rings were placed, in duplicate, on the surface of the test specimen and the polystyrene control specimen, and filled with the inoculated seedlayer medium.

The test and control specimens, provided with the seedlayer, were incubated for 4 days at 30°C. After 1, 2, 3, and 4 days of incubation of the test specimen the seedlayer was examined visually for the presence of visible (micro)colonies and growth-inhibition in comparison to the polystyrene control specimen.

The readings are recorded as:

- = no growth inhibition of the test organism, no deviations of numbers and size of the colonies developed in the culture medium;
- ± = moderate growth inhibition of the test organism, significant less and smaller colonies developed in the culture medium;
- + = complete growth inhibition of the test organism, no (micro)colonies developed in the culture medium.

3 Results and discussion

TNO Seedlayer Method

The results of the seedlayer tests for Marmoleum Topshield are summarized in table 2.1.

For the examined test specimen Marmoleum Topshield (TNO code 3119/04/ 0380) complete growth inhibition was found for the bacterial strains *Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus* RIVM 89-646, and *Listeria monocytogenes* ATCC 19114, i.e. no visible (micro)colonies developed in the seedlayers inoculated with these test organisms. After leaching of the test specimen by soaking with water complete growth inhibition was found for the same test organisms.

For the polystyrene control specimen clearly visible colonies developed in the seedlayer for each of the inoculated test organisms within the prescribed incubation period.

4 Conclusions

The obtained results of the antibacterial activity tests according to the TNO Seedlayer agardiffusion method indicate that the linoleum flooring type Marmoleum Topshield provides growth inhibition of *Staphylococcus aureus* bacteria, including MRSA strains, and *Listeria monocytogenes*.

The observed antibacterial activity of Marmoleum Topshield remains after soaking with water.

5 Signature

TNO Nutrition and Food Research



Dr. G.N.M. Huijberts, M.Sc.
Head, Department of Microbiology

Date: 8/8/04



B.J. Hartog, M.Sc.
Project Manager Microbiology

Date: 8-8-04

**Table 1 Marmoleum Topshield:
testorganisms used in the TNO Seedlayer test**

Forbo Linoleum BV., Krommenie (NL)

TNO project : 010.54087/01.10.01
Forbo ordernumber : 4500034258

| Nr. | Testorganisms | Characteristics |
|-----|---|---|
| 1 | <i>Staphylococcus aureus</i> strain ATCC 6538 | grampositive, coc-shaped bacterium, pathogen: a.o. furuncles; |
| 2 | <i>Staphylococcus aureus</i> strain RIVM 89-646 (MRSA) | hospital infections (MRSA-strains) |
| 3 | <i>Listeria monocytogenes</i> strain ATCC 19114 | grampositive, rod-shaped bacterium, pathogen: a.o meningitis |

Table 2.1 Marmoleum Topshield: microbiological growth inhibition according to the TNO Seedlayer Method**Forbo Linoleum BV, Krommenie**

TNO project : 010.54087/01.10.01

Forbo ordernumber : 4500034258, d.d. 17 June 2004

Date : July 14, 2004

| TNO code | Forbo code | remark | Test organisms | | |
|--------------|---------------------|---------------------------|---|-------------|---|
| | | | <i>Staphylococcus aureus</i> ATCC 6538 | RIVM 89-646 | <i>Listeria monocytogenes</i> ATCC 19114 |
| 3119/04/0380 | Marmoleum Topshield | untreated | +/+ | +/+ | +/+ |
| | | after soaking in water | +/+ | +/+ | +/+ |

../. = results of duplicate tests

- = no growth inhibition of the test organism
- ± = mild growth inhibition of the test organism
- + = complete growth inhibition of the test organism