



TROY CORPORATION

MICROBIOLOGY LABORATORY REPORT

**DENBER PAINTS LTD.
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**WET STATE PRESERVATION OF BACTERINOLL EMULSION
PAINT FOR INTERIOR, KITCHENS AND BATHROOMS**

PROJECT INITIATOR:

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**Report No.: T110305r TROY WS
Date: February 29, 2012**

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PROJECT BACKGROUND AND OBJECTIVE:

This study is to qualify Mergal K14PLUS and Mergal 758 as wet state preservatives for Bacterinoll.

Dry Film Fungicide Test and Antibacterial properties of the Bacterinoll paint film according to JIS Z2801 were reported separately

SAMPLE IDENTIFICATION:

TABLE 1: SAMPLE LABELS

SPNO	SPECIMEN
03	Bacterinoll
03.01	Bacterinoll + 0.04% Mergal K14PLUS
03.02	Bacterinoll + 0.05% Mergal K14PLUS
03.03	Bacterinoll + 0.1% Mergal 758

- Samples were received on November 11 2011.
- SPNO is the laboratory-assigned number.
- Sub-samples were prepared with the addition of the test microbicides as on Table 1.
- Additional sample description is on Table 2.

SUMMARY AND CONCLUSION:

At Arrival the paint sample was free of any microbial infection.

The Bacterinoll paint (sample as tested) is not sensitive to bacterial infection in the wet state. However, a safety wet state preservation with either Mergal K14 PLUS or Mergal 758 is highly recommended.\

A total of six inoculations were done.

The blank (SPNO 03) only showed susceptibility to the test bacteria after 5 massive infections. Mergal 758 and Mergal K14NPLUS proofed good efficacy at the tested use levels.

Test results are presented on Result Tables.

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All information and recommendations furnished are for guidance and are without guarantee. All preservatives should be field tested prior to use in actual manufacturing.

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RESULTS TABLES

TABLE 2: SAMPLE DESCRIPTION

SPNO	SPECIMEN	pH	COLOR	STATE
03	Bacterinoll	8.39	white	emulsion

TABLE 3: INITIAL MICROBIAL CONDITION

SPNO	SPECIMEN	STE BN	STE FN
03	Bacterinoll	0	0

STE BN= Sterility Check with Bacterial Nutrient

STE FN= Sterility Check with Fungal Nutrient

GROWTH RATING SYSTEM:

- 0: No colonies found
- 1: 1-10 colonies found
- 2: 11-100 colonies found
- 3: More than 100 colonies found recognizable; Streaks not fully overgrown
- 4: Streaks fully overgrown

- B: Bacteria
- M: Molds
- Y: Yeasts
- NT: Not Tested

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TABLE 4: WET STATE PRESERVATION AGAINST BACTERIA

SPNO	SPECIMEN	1/7d	2/7d	3/7d	4/1d	4/2d	4/7d	5/7d
03	Bacterinoll	0	0	0	4	4	0	4
03.01	Bacterinoll + 0.04% Mergal K14PLUS	0	0	0	0	0	0	2
03.02	Bacterinoll + 0.05% Mergal K14PLUS	0	0	0	0	0	0	1
03.03	Bacterinoll + 0.1% Mergal 758	0	0	0	0	0	0	2

Legend:

1/7d: 1st Inoculation /Incubation time 7 days

2/7d: 2nd Inoculation /Incubation time 7 days

3/1d to 3/7d: 3rd Inoculation /Incubation time 1 day to 7 days

4/1d to 4/7d: 4th Inoculation /Incubation time 1 day to 7 days

5/7d: 5th Inoculation /Incubation time 7 days

6/1d to 6/7d: 6th Inoculation /Incubation time 1 day to 7 days

GROWTH RATING SYSTEM:

0: No colonies found

1: 1-10 colonies found

2: 11-100 colonies found

3: More than 100 colonies found recognizable; Streaks not fully overgrown

4: Streaks fully overgrown

M: Molds

Y: Yeasts

NT: Not Tested

The rating 7 days after inoculation indicates the preservation quality.

The rating after 1 and 2 days indicates the speed of kill of inoculated bacteria.

The paint must have been massively inoculated with adapted test bacteria and a total more than 20% dilution before bacteria could establish in the unfortified paint.

Therefor the test was extended to 5 inoculations.

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MATERIALS AND TEST PROCEDURES:

The samples were tested for **Initial Microbial Condition** and **Wet State Preservative Properties** in accordance with the TROY test methods.

Initial Microbial Condition

The samples were streaked onto TSA and MEA and incubated for one week at 30° C and 28°C, respectively.

Culture media: Tryptic Soy Agar (TSA)
 Malt Extract Agar (MEA)

Wet State Preservative Properties

Test of Resistance to Bacterial Growth

The samples were inoculated with 1.0% of a mixture of the bacterial cultures listed below. The inoculated samples were incubated at room temperature and streaked onto TSA seven days after the first and second inoculations. For the third week, the inoculated samples were streaked onto TSA one, two and seven days after inoculation.

Rating schemes as indicated at the result tables.

For the bacterial challenge test the preservation quality is indicated by the rating of the 3rd inoculation evaluated after a 7 day incubation period. The speed of kill of the inoculated bacteria is indicated by the rating after 1 and 2 days (3rd Inoculation).

Test organisms for bacterial test: 1.0% of mixed cultures of the following:

- | | |
|-----------------------------------|------------------------------------|
| 1 - <i>Alcaligenes faecalis</i> | 5 - <i>Proteus hauseri</i> |
| 2 - <i>Enterobacter aerogenes</i> | 6 - <i>Pseudomonas aeruginosa</i> |
| 3 - <i>Escherichia coli</i> | 7 - <i>Pseudomonas fluorescens</i> |
| 4 - <i>Myroides odoratus</i> | 8 - <i>Pseudomonas putida</i> |
| | 9 - <i>Pseudomonas stutzeri</i> |

Culture density: $>10^8$ bacterial cells/ mL = 5.5×10^8 (1 to 9 bacteria)

For the blank with no or poor growth (rating 1-2) after the first inoculation, an adapted inoculum was used for the succeeding inoculations. The "adapted inocula" are microbes from the standard infection, grown in a 1:10 dilution of the unpreserved test material. Diluent was Nutrient Broth. This then was used to inoculate further the samples at 10% on succeeding inoculations.

All organisms are held on culture in the Microbiology Laboratory.

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