TROY CORPORATION

MICROBIOLOGY LABORATORY REPORT

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ANTI-MICROBIAL EQUIPMENT OF BACTERINOLL EMULSION PAINT FOR INTERIOR, KITCHENS AND BATHROOMS
TEST OF THE FUNGAL RESISTANCE ACCORDING TO TROY AGAR DIFFUSION TEST METHOD
TEST OF THE ANTIBACTERIAL SURFACE PROPERTIES ACCORDING TO ISO22196 (JIS Z2801)

PROJECT INITIATOR:
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CONFIDENTIAL

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PROJECT BACKGROUND AND OBJECTIVE:

Bacterinoll paint is sold as kitchen and bathroom paint equipped with Parmetol DF19
This project evaluates the antifungal effects of the full spectrum fungicide algaecide Polyphase 689, which could also be used for exterior paints and Polyphase PW40.
Other microbicides were tested as additional options as outlined on this report

BACTERINOLL is also sold as anti-bacterial paint.

This report is for the antifungal effect of the Bacterinoll coating as well as the anti-bacterial effect of the surface tested according to ISO22196 (JIS Z2801)

Separate report was issued for the Wet State Preservation tests.

SAMPLE IDENTIFICATION:

TABLE 1: SAMPLE LABELS

SPNO	INDICATION	NOTES
03	Bacterinoll	As received from company Denber
03.01	Bacterinoll + 0.04% Mergal K14PLUS	For wet state preservation test only
03.02	Bacterinoll + 0.05% Mergal K14PLUS	For wet state preservation test only
03.03	Bacterinoll + 0.1% Mergal 758	For wet state preservation test only
03.04	Bacterinoll + 0.12% Polyphase PW40	Fungicide
03.05	Bacterinoll + 0.2% Parmetol DF19	Parmetol DF19 incumbant fungicide-algaecide ref A7034
03.06	Bacterinoll + 0.12% Polyphase 7026	P7026 was tested: this is the Fungicide part of 0.5% Polyphase 689
03.07	Bacterinoll + 0.6% Polyphase 2167	
03.09	Bacterinoll + 0.5% Troysan 1050	
04	Positive control for JIS Z2801	Architectural paint which was demonstrated to be susceptible at JIS Z2801 Test – ref.: T110099-03

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- 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:

Antibacterial Equipment of Bacterinoll – Test according to ISO22196 (JIS Z2801)

Bacterinoll as received (SPNO 03) was highly susceptible to both test bacteria. Additions of Polyphase 2167, and Troysan 1050 imparted excellent anti-bacterial properties to the Bacterinoll paint film.

Test of the Resistance to Fungal Growth:

0.2% Parmetol DF19 does not impart resistance to fungal growth to Bacterinoll. The equivalent of 0.5% Polyphase 689 worked well against *Aspergillus niger* but failed the test against *Alternaria alternata*

Excellent protection against all test fungi was provided by 0.12% Polyphase PW40 or 0.6% Polyphase 2167

Test results are presented on Result Table.

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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

RESULTS TABLES

TABLE 2: SAMPLE DESCRIPTION

SPNO	SPECIMEN	pH	COLOR	STATE
03	Bacterinoll	8.39	white	emulsion

TABLE 3: EVALUATION OF THE ANTI-BACTERIAL EFFECT OF PAINT FILM (JIS Z2801-TR)

SPNO	INDICATION	CFU recovered *Ec* L 0.5h	Log reduction *Escherichia coli*	CFU recovered *Sa* L 0.5h	Log reduction *Staphylococcus aureus*
03	Bacterinoll	EXP 7	basis	EXP 6	basis
03.07	**Bacterinoll + 0.6% Polyphase 2167**	0	**>2**	0	**>2**
03.09	**Bacterinoll + 0.5% Troysan 1050**	0	**>2**	0	**>2**
04	Positive control T110099-03	EXP 7	+Control of method	EXP 7	+Control of method

Test Organisms: ***Ec***=Escherichia coli; ***Sa***= *Staphylococcus aureus*

Leaching treatments: L 0.5h = 0.5 hour in water

CFU: number of colony-forming units (bacterial cells) recovered after 24h incubation (for the efficacy calculation the logarithm of the cfu is formed (example EXP 7 means 10 0000000 cfu, which logarithm log (cfu) is 7)

Basis: the unfortified sample, which is the basis of log reduction calculation of the following fortified samples

NS: not suitable, as the basis (the unfortified test film) did not support bacterial growth

Result Interpretation (Log reduction):
>2 = passed
<2 = failed

0.5% Troysan 1050 or 0.6% Polyphase 2167, respectively imparted to Bacterinoll complete control of the test bacteria. The fortified paint exceeded the criteria of the Japanese Standard JIS Z2801 for a at least log 2 reduction (>99% kill) of the test bacteria.

TABLE 4: ANTI-FUNGAL TESTS (Troy Agar Diffusion Method) AD TR

SPNO	SPECIMEN	Aa L 1h	An+Pf L 1h
03	Bacterinoll	4	4
03.04	Bacterinoll + 0.12% Polyphase PW40	1	0Z
03.05	Bacterinoll + 0.2% Parmetol DF19	4	4
03.06	Bacterinoll + 0.12% Polyphase 7026	4	1
03.07	Bacterinoll + 0.6% Polyphase 2167	1	0Z

Test organisms: *A a* = *Alternaria alternata*
A n+ Pf = *Aspergillus niger* + *Penicillium funiculosum*

Leaching: **L 1h = 1hour in water**

GROWTH RATING SYSTEM:

0Z: No growth on the specimen, zone of inhibition may be present
0: No growth on the specimen surface
1: less than 10% of the specimen surface covered
2: less than 30% of the specimen surface covered
3: less than 60% of the specimen surface covered
4: more than 60% of the specimen surface covered or specimen surface totally overgrown

0.12% Polyphase 7026 is the fungicide part equivalent of 0.5% Polyphase 689. Polyphase 689 has an additional algaecide, which however does not have a function in interior applications

MATERIALS AND TEST PROCEDURES:

The samples were tested for **Anti-fungal Preservative Properties** in accordance with the TROY Agar Diffusion test methods.

Anti-fungal Preservative Properties:
A layer of sample (300µm) was applied onto filter paper, and air-dried for 2 days.
Leaching treatments were done as specified on Result Tables.

The prepared samples were then placed on the seeded Malt Extract Agar and incubated for 2 weeks at 28°C. The fungal inocula used were *Alternaria alternata;* and *Aspergillus niger* + *Penicillium funiculosum.*

All prepared samples were sterilized by γ-radiation at a level of ±35 kGy prior to the test with microorganisms.

The samples were tested for **Anti-bacterial Dry Film Properties** basically in accordance with the Japanese Standard Test JIS Z2801.

The sample was applied onto a Black Leneta then air-dried for 7 days.
The coated sample was leached as specified on Result Table and air-dried for 2 days.
Each pre-cut specimen (50 x 50 mm) was placed in a plastic container then separately inoculated with *Escherichia coli* and *Staphylococcus aureus.* The inoculated samples were then covered with plastic and incubated at 35C for 24 hours. Recovery was done with 10mL Tryptic Soy Broth and with streaking onto Nutrient Agar plates. Incubation of the inoculated agar was at 30C for 24 hours.

A positive control was also included to check the validity of the method, particularly the viability of the test bacteria. This was the sample previously demonstrated as very susceptible to the test organisms.

All organisms are held on culture in the Microbiology Laboratory.

Annex 1: Photos Test of the Resistance to Fungal Growth – Agar Diffusion Test