



d technology Pvt. ltd.

Unit No-19, Building No-F-8, Bhumi World Industrial Park,
Opp. Tata Amantra, Pimpas, Mumbai-Nashik Highway, Thane-421302

Test Report

Report No: 19-20/10013

Report Date: 1st July, 2019

Prepared For- Mr. Umesh Tripathi
SANOSIL BIOTECH PVT. LTD.
Warden House, 1st Floor,
Sir P.M. Road, Fort,
Mumbai - 400 001

Prepared By- Ms. Darshana Bagwe
Executive-Techno commercial,
d technology Pvt. Ltd

Objective:

To estimate the antimicrobial efficacy of Virosil against standard microbial cultures.

Sample Details:

Sample Type	Name	Batch No	Mfg Date	Expiry Date	Quantity
Disinfectant	Virosil Chlorine Free Fumigant	VPS19059	May 2019	April 2021	5 Litres

Test Method: As per client's In-house protocol

Concentration: 20%

Test Organisms:

- 1) *Bacillus subtilis* ATCC 6633
- 2) *Bacillus cereus* ATCC 6630
- 3) *Aspergillus niger* ATCC 16404

Contact Time:

- a. 30 minutes
- b. 60 minutes

Clients In-House Protocol summary:

Culture preparation:

- a) The selected organisms were subcultured on Soyabean Casein Digest agar one day prior to analysis. These plates were incubated at 30^oC-35^oC for 24 hours.
- b) Post incubation the culture suspensions were prepared in 9ml St. Saline and serially diluted upto 10⁻⁸ to 10⁻⁹
- c) 0.1ml and 1 ml was surface spread and pour plated respectively from each dilution tube on Soyabean Casein Digest agar and incubated at 30^oC-35^oC for 24 hours. Both were stored at 2^oC-8^oC until further use.

1. Selection of culture tube:

- a) Post incubation the tube containing 1000-10000 cfu/ml (refrigerated tubes) were selected.

2. Procedure for positive control:

- a) 100 fold dilutions were performed from the selected tube for both spread spread and pour plate method i.e. 0.1ml from the tube in 9.9ml saline
- b) 0.1ml was then taken from these tubes and filtered in a membrane filtration assembly using 0.1% Peptone and 0.1% Tween 80.

3. Procedure for test:

- a) 0.1ml from the selected tube of spread plate and pour plate method was taken and added it to 9.9ml of diluted virosil (20%) for required contact time .i.e. 30 minutes and 60 minutes.
- c) Post contact time the filtered contents from the membrane were washed with 2X 100ml 0.1% Peptone and 0.1% Tween 80.
- b) Then 2 minutes of contact was given with 9ml Dey Engley neutralizing broth and then filtered again.
- d) Finally the filtered contents were washed again with 100ml of 0.1% Peptone and 0.1% Tween 80 and the membrane paper was placed on Soyabean Casein Digest agar.
- d) These plates were incubated at 30^oC-35^oC for 24 hours and counts were recorded post incubation.

Note: Sabouraud Dextrose Agar was used for *Aspergillus niger* ATCC 16404 and culture was serially diluted upto 10⁻⁷ to 10⁻⁸

Results:

1. *Bacillus subtilis* ATCC 6633

Spread plate count

TEST ORGANISM	Trial	Control	Avg cfu /ml	Log value
<i>Bacillus subtilis</i> ATCC 6633	N=1	Positive control	6.40×10^1	1.8061

TEST ORGANISM	Trial	Initial culture count	Log value	Time of exposure	Avg cfu /ml	Log value	Log reduction	Percent reduction
<i>Bacillus subtilis</i> ATCC 6633	N=1	6.80×10^4	4.8325	30 minutes	$>3.30 \times 10^2$	>2.5185	<2.3140	-
				60 minutes	2.20×10^1	1.3424	3.4901	99.967%

Pour plate count

TEST ORGANISM	Trial	Control	Avg cfu /ml	Log value
<i>Bacillus subtilis</i> ATCC 6633	N=1	Positive control	1.00×10^1	1.0000

TEST ORGANISM	Trial	Initial culture count	Log value	Time of exposure	Avg cfu /ml	Log value	Log reduction	Percent reduction
<i>Bacillus subtilis</i> ATCC 6633	N=1	7.50×10^3	3.8750	30 minutes	5.50×10^1	1.7403	2.1347	99.266%
				60 minutes	$<1.00 \times 10^1$	<1.0000	>2.8750	>99.866%

2. *Bacillus cereus* ATCC 6630

Spread plate count

TEST ORGANISM	Trial	Control	Avg cfu /ml	Log value
<i>Bacillus cereus</i> ATCC 6630	N=1	Positive control	7.00 x 10 ¹	1.8450

TEST ORGANISM	Trial	Initial culture count	Log value	Time of exposure	Avg cfu /ml	Log value	Log reduction	Percent reduction
<i>Bacillus cereus</i> ATCC 6630	N=1	7.15 x 10 ⁴	4.8543	30 minutes	7.90 x 10 ¹	1.8976	2.9567	99.890%
				60 minutes	1.80 x 10 ¹	1.2552	3.5991	99.975%

Pour plate count

TEST ORGANISM	Trial	Control	Avg cfu /ml	Log value
<i>Bacillus cereus</i> ATCC 6630	N=1	Positive control	1.10 x 10 ¹	1.0413

TEST ORGANISM	Trial	Initial culture count	Log value	Time of exposure	Avg cfu /ml	Log value	Log reduction	Percent reduction
<i>Bacillus cereus</i> ATCC 6630	N=1	7.85 x 10 ³	3.8948	30 minutes	3.20 x 10 ¹	1.5051	2.3897	99.592%
				60 minutes	6	0.7781	3.1167	99.924%

3. *Aspergillus niger* ATCC 16404

Spread plate count

TEST ORGANISM	Trial	Control	Avg cfu /ml	Log value
<i>Aspergillus niger</i> ATCC 16404	N=1	Positive control	2.90 x 10 ¹	1.4623

TEST ORGANISM	Trial	Initial culture count	Log value	Time of exposure	Avg cfu /ml	Log value	Log reduction	Percent reduction
<i>Aspergillus niger</i> ATCC 16404	N=1	3.15 x 10 ⁴	4.4983	30 minutes	<10	<1.0000	>3.4983	>99.968%
				60 minutes	<10	<1.0000	>3.4983	>99.968%

Pour plate count

TEST ORGANISM	Trial	Control	Avg cfu /ml	Log value
<i>Aspergillus niger</i> ATCC 16404	N=1	Positive control	4.00 x 10 ¹	1.6020

TEST ORGANISM	Trial	Initial culture count	Log value	Time of exposure	Avg cfu /ml	Log value	Log reduction	Percent reduction
<i>Aspergillus niger</i> ATCC 16404	N=1	3.70 x 10 ³	3.5682	30 minutes	<10	<1.0000	>2.5682	>99.730%
				60 minutes	<10	<1.0000	>2.5682	>99.730%

Conclusion:

The efficacy of submitted disinfectant sample Virosil at concentration of 20% in terms of percent reduction (%) is given below:

Test Organisms	Spread plate count		Pour plate count	
	30 minutes	60 minutes	30 minutes	60 minutes
<i>Bacillus subtilis</i> ATCC 6633	-	99.967%	99.266%	>99.866%
<i>Bacillus cereus</i> ATCC 6630	99.890%	99.975%	99.592%	99.924%
<i>Aspergillus niger</i> ATCC 16404	>99.968%	>99.968%	>99.730%	>99.730%

Checked by

Name: Ms. Darshana Bagwe

Designation: Executive-Techno commercial

Authorized Signatory

Name: Dr. Deepa Bhajekar

Designation: Managing Director

Note:

1. The results reported relate only to the sample(s) tested.
2. This test report, in full or in part, shall not be reproduced, used for any legal action, without prior written approval of laboratory.
3. The tests marked with #are subcontracted.
4. The Report no. with suffix R- Revised Report.

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