

EFFICACY DATA FOR BIO-CLEAN

(EPA Reg. No. 1839-169)

VIRUCIDAL DATA:

Test Methods:

*U.S E.P.A Pesticide Assessment Guidelines, Subdivision G: Product Performance, Section 91-2(f), and Section 91-30 (d),(e), November, 1982.

+Protocols for Testing the Efficacy of Disinfectants against Hepatitis B Virus (HBV) (EPA, Federal Register, Vol.65, No.166, 8/25/2000, p.51828).

*Protocol for testing Disinfectants against Hepatitis C Virus using Bovine Viral Diarrhea Virus as approved by the U.S EPA on August 15, 2002.

TEST CONDITIONS: 10 minute contact time, 5% organic soil load, sterile glass petri dishes,
400 ppm hard water, 21-24°C exposure temperature, 2 oz/gal dilution

Results:

<u>Test Organism</u>	<u>Sample</u>	<u>Titer Reduction</u>	
*Avian Influenza A virus (H3N2) (Avian Reassortant) (ATCC VE-2072)	A B	≥4.25 log ₁₀	>4.25 log ₁₀
*Avian Influenza Virus, Type A (Turkey/WIS/66) (H9N2)	A B	≥4.0 log ₁₀	≥4.0 log ₁₀
*Bovine Rhinotracheitis, strain LA (ATCC VR-188)	A B	≥5.0 log ₁₀	≥5.0 log ₁₀
*Bovine Viral Diarrhea Virus (BVDV)	A B	5.9 log ₁₀	5.9 log ₁₀
*Canine Distemper Virus, strain Lederle (ATCC VR-128)	A B	≥6.25 log ₁₀	≥6.25 log ₁₀
*Feline Picornavirus, strain FRV (ATCC VR-649)	A B	≥4.25 log ₁₀	≥4.25 log ₁₀
+Hepatitis B Virus (HBV) (Duck Hepatitis B Virus-DHBV)	A B	4.5 log ₁₀	4.7 log ₁₀
*Hepatitis C Virus (HCV) (Bovine Viral Diarrhea Virus-BVDV)	A B	5.9 log ₁₀	5.9 log ₁₀
*Herpes Simplex Type 1 (ATCC VR-260)	A B	≥5.01 log ₁₀	≥5.0 log ₁₀
*Herpes Simplex Type 2 (ATCC VR-734)	A B	≥6.0 log ₁₀	≥6.0 log ₁₀
*Human Coronavirus (ATCC VR-740, Strain 229E)	A B	≥3.0 log ₁₀	≥3.0 log ₁₀
*Human Immunodeficiency Virus, HTLV-IIIrf, strain of HIV-1 (associated with AIDS)	A B	≥3.5 log ₁₀	≥3.5 log ₁₀
*Human Immunodeficiency Virus type 2 (HIV-2), strain CBL-20	A B	≥3.25 log ₁₀	≥3.25 log ₁₀
*Influenza A ₂ strain Hong Kong (ATCC VR-544)	A B	≥4.25 log ₁₀	≥4.25 log ₁₀
*Porcine Respiratory & Reproductive Syndrome Virus (PRRSV) strain NVSL	A B	≥5.0 log ₁₀	≥5.0 log ₁₀
*Pseudorabies strain Aujeszky (ATCC VR-135)	A B	≥5.25 log ₁₀	≥5.25 log ₁₀
*Rabies Virus (attenuated CDC ERA strain)	A B	3.0 log ₁₀	3.0 log ₁₀
*Rotavirus, strain SA-11 (ATCC VR-899)	A B	4.5 log ₁₀	4.5 log ₁₀
*SARS Associated Coronavirus (ZeptoMetrix)	A B	3.03 log ₁₀	3.03 log ₁₀
*Vaccinia, strain WR (ATCC VR-119)	A B	≥5.5 log ₁₀	≥5.5 log ₁₀

Conclusion: Under the conditions of this investigation, Bio-Clean, Neutral Disinfectant Cleaner was **virucidal** for Avian Influenza A Virus (H3N2), Avian Influenza Virus, Type A (H9N2), Bovine Rhinotracheitis, strain LA, Canine Distemper Virus, strain Lederle, Feline Picornavirus, strain FRV, Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Herpes Simplex Type 1, Herpes Simplex Type 2, Human Coronavirus, Human Immunodeficiency Virus (HIV-1), Human Immunodeficiency Virus type 2 (HIV-2), Influenza A₂, Porcine Respiratory & Reproductive Syndrome Virus (PRRSV), Pseudorabies strain Aujeszky, Pseudorabies, Rotavirus, SARS Associated Coronavirus and Vaccinia according to criteria established by the U.S Environmental Protection Agency for registration and labeling of disinfectant product as a virucide.

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MILDEW FUNGISTATIC DATA:

Test Method: Hard Surface Mildew Fungistatic Test

Test Organism: *Aspergillus niger* (ATCC 6275)

Test Conditions: 400 ppm hard water, ceramic tile carriers, 2 oz/gal dilution

Results:

<u>Sample</u>	<u>No. of Exposed Tiles</u>	<u>No. of Tiles Showing Growth</u>
A	10	0
B	10	0
Control	10	10

Conclusion:

Under the conditions of this investigation, Bio-Clean Neutral Disinfectant Cleaner was **fungistatic** for *Aspergillus niger* according to criteria established by the U.S Environmental Protection Agency for registration and labeling of a disinfectant product as a fungistat.

FUNGICIDAL DATA:

Test Method: AOAC fungicidal Activity of Disinfectants

Test Conditions: 5% organic soil load 20°C exposure temperature
200 ppm hard hard water, 2 oz/gal dilution

<u>Test Organism</u>	<u>Sample</u>	<u>Exposure Time (min.) vs. Growth</u>		
		<u>5</u>	<u>10</u>	<u>15</u>
Trichophyton mentagrophytes (ATCC 9533)	A	+	0	0
	B	+	0	0
Candida albicans (ATCC 10231)	A	0	0	0
	A	0	0	0

Conclusions:

Under the conditions of this investigation, Bio-Clean Neutral Disinfectant Cleaner was **fungicidal** for *Trichophyton mentagrophytes* and *Candida albicans* according to criteria established by the U.S Environmental Protection Agency for registration and labeling of a disinfectant product as a fungicide.

DISINFECTION DATA:

Test Method: AOAC Use Dilution

Test Conditions: 10 minute contact time 5% organic soil load, stainless steel carrier substrates 400 ppm hard water, 20°C exposure temperature, 2 oz/gal dilution [with the exception of *Xanthomonas axonopodis* pathovar citri, dilution ratio is indicated by organism]

Results:

<u>Test Organism</u>	<u>Sample</u>	<u>No. of Carriers</u>	
		<u>Expose</u>	<u>Positive</u>
Staphylococcus aureus (ATCC 6538)	A	60	1
	B	60	1
	C	60	1
Salmonella enterica subsp. enterica serotype choleraesuis (ATCC 10708)	A	60	0
	B	60	0
	C	60	1
Pseudomonas aeruginosa PRD-10 (ATCC 15442)	A	60	0
	B	60	0
	C	60	1
Bordetella bronchiseptica (ATCC 31437)	A	10	0
	B	10	0
Community Associated Methicillin Resistant Staphylococcus aureus (CA-MRSA) (NRS 123, Genotype USA400)	A	10	0
	B	10	0
	C	10	0
Community Associated Methicillin Resistant Staphylococcus aureus (CA-MRSA) (NRS 384, Genotype USA300)	A	10	0
	B	10	0
	C	10	0
Corynebacterium ammoniagens (ATCC 6871)	A	10	0
	B	10	0
Enterobacter aerogenes (ATCC 13048)	A	10	0
	B	10	0
Enterobacter cloacae (ATCC 2335)	A	10	0
	B	10	0
Enterobacter cloacae (clinical isolate)	A	10	0
	B	10	0
Enterococcus faecalis (ATCC 19433)	A	10	0
	B	10	0
Enterococcus faecalis (clinical isolate)	A	10	0
	B	10	0

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DISINFECTATION DATA (continued)

<u>Test Organism</u>	<u>Sample</u>	<u>No. of Carriers</u>	
		<u>Expose</u>	<u>Positive</u>
Escherichia coli (ATCC 11229)	A	10	0
	B	10	0
Escherichia coil (clinical isolate)	A	10	0
	B	10	0
Fusobacterium necrophorum (ATCC 27852)	A	10	0
	B	10	0
Klebsiella pneumoniae subsp. pneumoniae (ATCC 13883)	A	10	0
	B	10	0
Lactobacillus casei subsp. rhamnosus (ATCC 7469)	A	10	0
	B	10	0
Listeria monocytogenes (ATCC 35152)	A	10	0
	B	10	0
Methicillin Resistant Staphylococcus aureus (MRSA) (ATCC 33592)	A	10	0
	B	10	0
Pasteurella multocida (ATCC 7707)	A	10	0
	B	10	0
Proteus mirabilis (ATCC 9921)	A	10	0
	B	10	0
Proteus mirabilis (ATCC 25933)	A	10	0
	B	10	0
Proteus vulgaris (ATCC 13315)	A	10	0
	B	10	0
Salmonella enterica subsp. enterica serotype paratyphi B (ATCC 8759)	A	10	0
	B	10	0
Salmonella enterica subsp. enterica aerotype pullorum (ATCC 9120)	A	10	0
	B	10	0
Salmonella enterica subsp. enterica serotype typhi (ATCC 6539)	A	10	0
	B	10	0
Salmonella enterica subsp. enterica serotype typhimurium (ATCC 14028)	A	10	0
	B	10	0
Serratia marcescens (ATCC 8100)	A	10	0
	B	10	0
Shigella dysenteriae (ATCC 12180)	A	10	0
	B	10	0

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DISINFECTATION DATA (continued)

<u>Test Organism</u>	<u>Sample</u>	<u>No. of Carriers</u>	
		<u>Expose</u>	<u>Positive</u>
Shigella flexneri Type 2b (ATCC 12022)	A	10	0
	B	10	0
Shigella sonnei (ATCC 25931)	A	10	0
	B	10	0
Staphylococcus aureus subsp. aureus (ATCC 33592)	A	10	0
	B	10	0
Staphylococcus aureus (clinical isolate)	A	10	0
	B	10	0
Staphylococcus epidermidis (ATCC 29641)	A	10	0
	B	10	0
Staphylococcus epidermidis (clinical isolate)	A	10	0
	B	10	0
Streptococcus pyogenes Group A (ATCC 19615)	A	10	0
	B	10	0
Streptococcus pyogenes (clinical-flesh eating strain, BIRD M3)	A	10	0
	B	10	0
Vancomycin Resistant Enterococcus faecalis (VRE) (ATCC 51575)	A	10	0
	B	10	0
Vancomycin Intermediate Resistant Staphylococcus aureus (VISA) (HIP 5863)	A	10	0
	B	10	0
Xanthomonas maltophilia (clinical isolate)	A	10	0
	B	10	0
Xanthomonas axonopodis pathovar citri at a dilution ratio of 1:27 (2000 ppm active quat)	A	10	0
	B	10	0

Conclusion:

Under the conditions of these investigations, Bio-Clean Neutral Disinfectant Cleaner demonstrated **disinfectant** activity against Staphylococcus aureus, Salmonella enterica subsp. enterica serotype choleraesuis, Pseudomonas aeruginosa, Bordetella bronchiseptica, Community Associated Methicillin Resistant Staphylococcus aureus (CA-MRSA) (NRS 123, Genotype USA400), Community Associated Methicillin Resistant Staphylococcus aureus (CA-MRSA) (NRS 384, Genotype USA300), Corynebacterium ammoniagenes, Enterobacter aerogenes, Enterobacter cloacae, Enterococcus faecalis, Escherichia coli, Fusobacterium necrophorum, Klebsiella pneumoniae subsp. pneumoniae, Lactobacillus casei subsp. rhamnosus, Listeria monocytogenes, Methicillin Resistant Staphylococcus aureus (MRSA) Pasteurella multocida, Proteus mirabilis (ATCC 9921), Proteus mirabilis (ATCC 25933), Proteus vulgaris, Salmonella enterica subsp. enterica serotype paratyphi B, Salmonella enterica subsp. enterica serotype pullorum, Salmonella enterica subsp. enterica serotype typhi, Salmonella enterica subsp. enterica serotype typhimurium, serratia marcescens, shigella dysenteriae, Shigella flexneri Type 2b, Shigella sonnei, Staphylococcus aureus subsp. aureus, Staphylococcus epidermidis, Streptococcus pyogenes Group A, Streptococcus pyogenes (clinical-flesh eating strain, BIRD M3), Vancomycin Resistant Enterococcus faecalis (VRE), and Vancomycin Intermediate Resistant Staphylococcus aureus (VISA), according to criteria established by the U.S Environmental Protection Agency for registration and labeling of a disinfectant product as a bactericide.

Bio-Clean Neutral Disinfectant Cleaner also demonstrated disinfectant activity against the following antibiotic resistant clinical isolates: Enterobacter cloacae, Enterococcus faecalis, Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, and Xanthomonas maltophilia.

At a dilution ratio of 1:27 (2000 ppm active quat), Bio-Clean Neutral Disinfectant Cleaner demonstrated **disinfectant** activity against Xanthomonas axonopodis pathovar citri (Citrus Canker Disease)

BACTERICIDAL STABILITY DATA OF USE-SOLUTION:

Test Method: Use Dilution

Test Conditions: 5% organic soil load, 10 minute contact time, stainless steel carrier substrates, 400 ppm hard water. 20°C exposure temperature, 2 oz/gal dilution

Storage Conditions: Sealed container at room temperature

Results:

<u>Test Time</u>	<u>Sample</u>	<u>Test Organism</u>	<u>No. of Carrier</u>	
			<u>Exposed</u>	<u>Positive</u>
Zero Time	A	Staphylococcus aureus (ATCC 6538)	10	0
		Salmonella enterica (ATCC 10708)	10	0
		Pseudomonas aeruginosa (ATCC 15442)	10	0
	B	Staphylococcus aureus (ATCC 6538)	10	0
		Salmonella enterica (ATCC 10708)	10	0
		Pseudomonas aeruginosa (ATCC 15442)	10	0
1 Week	A	Staphylococcus aureus (ATCC 6538)	10	0
		Salmonella enterica (ATCC 10708)	10	0
		Pseudomonas aeruginosa (ATCC 15442)	10	0
	B	Staphylococcus aureus (ATCC 6538)	10	0
		Salmonella enterica (ATCC 10708)	10	0
		Pseudomonas aeruginosa (ATCC 15442)	10	0
Week 2	A	Staphylococcus aureus (ATCC 6538)	10	0
		Salmonella enterica (ATCC 10708)	10	0
		Pseudomonas aeruginosa (ATCC 15442)	10	0
	B	Staphylococcus aureus (ATCC 6538)	10	0
		Salmonella enterica (ATCC 10708)	10	0
		Pseudomonas aeruginosa (ATCC 15442)	10	0
Week 3	A	Staphylococcus aureus (ATCC 6538)	10	0
		Salmonella enterica (ATCC 10708)	10	0
		Pseudomonas aeruginosa (ATCC 15442)	10	0
	B	Staphylococcus aureus (ATCC 6538)	10	0
		Salmonella enterica (ATCC 10708)	10	0
		Pseudomonas aeruginosa (ATCC 15442)	10	0
Week 5	A	Staphylococcus aureus (ATCC 6538)	10	0
		Salmonella enterica (ATCC 10708)	10	0
		Pseudomonas aeruginosa (ATCC 15442)	10	0
	B	Staphylococcus aureus (ATCC 6538)	10	0
		Salmonella enterica (ATCC 10708)	10	0
		Pseudomonas aeruginosa (ATCC 15442)	10	0

Conclusion:

The results of this investigation show that a 2 oz/gal use dilution of Bio-Clean Neutral Cleaner will demonstrate disinfectant efficacy against Staphylococcus aureus, Salmonella enterica, and pseudomonas aeruginosa for up to 4 weeks in accordance with criteria established by the U.S Environmental Protection Agency for registration and labeling of a disinfectant product as a bactericide.