MAR 2 1 :cos Contact Time Required to Kill The Disease Organisms Listed (0.5 ppm lodine, pH 7.5, 20-65 C) SGS U.S. Testing Company Inc. 20238 BACTERIA MENIMUM CONTACT TIME 3/18/9 Evaluation of antibacterial properties of a water purifier system. SUBJECT: Escherichia coli 50 seconds ... Salmonella typhosa P-4 1 minute Sample received from client on 2/29/96. Identified the sample as: SAMPLE ID: water bottle Salmonella typhose P-5 ... I minute ** Salmonella typhose P-10 I minute Sample is a one pint clear plastic water bottle with blue cap, Inside the ... Salmonella pararvphi P-12 I minute neck of the bottle is "shot glass" shaped filter composed of a Salmonella schottmulleri P-3 ... 2 minutes composite includeing lodine resin. ... Salmonella typhimurium P-611 5 minutes Shigella flexneri P-7 2 minutes Antibacterial testing was based on methods outlined in United States TEST .. Shigella dysentariae 11 P-8 2 minutes Environmental Protection Agency's "Guide Standard and Protocol for ... Shigella sonnei 1 P-9 2 minutes ഗ Testing Microbiological Water Purifiers.", Revised April 1987. ++ Streptococcus fecalis E-40 2 minutes ... Staphylococcus aureus 50 seconds NOTE: this study is only a screen of antibacterial effectiveness for thi: PROCEDURE: Staphylococcus epidermidis 1 minute product, and does not offer data on viral or protozoan efficacy as required by above referenced document. VIRUS MINIMUM CONTACT TIME EL. March 12 -March 18, 1996 \bigcirc Poliovirus Type 1 . 9 minutes EST DATES: MINIMUM CONTACT TIME CYSTS (a: 1 ppm Iodine) r EST PROCEDURE OUTLINE: **TEST SPECIES:** Klebsiella terrigena ATCC 33257 Entamoeba Histolytica 30 minutes Ω TEST WATER: EPA Test Water #2 One liter of deionized water was amended as follows: T \mathbf{m} MAR Z 1 1905 pH adjusted to 4.97 with HCI. 1.) Total Organic Carbon (TOC) adjusted to 17.0 mg/L with 2.1 humic acid. Turbidity adjusted to 34 NTU with AC Spark Plug Dust. 3.) SGS U.S. Testing Company Inc. TDS adjusted to 1450 mg/L with sea salts. 4.) 5.) Temperature adjusted to 4°C by placing in refrigerator assaic Avenue 202381 for 4 hours. Fairfield, NJ 07004 Date: 3/18/96 Tel: 201 575 5252 CONTACT TIME: 0, 1.0, 2.5, and 5.0 minutes Fax: 201 244 1823 Screening Study to Assess Antibacterial Properties RECOVERY MEDIA: Violet Red Bile Agar (VRBA) of Water Bottle NEUTRALIZER: 0.1 Normal Sodium thiosulfate Test Report No. 202381 Page 2 of 4 Signed for the Company by: Prepared by: March 18, 1996 Daniel Drozdo Inthon. Grilli, MS Director Biological Services Manager, Microbiology

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ST PROCEDURE OUTLINE;

- One liter of test water was inoculated with one ml of Klebsiella tenigena so that the final concentration was approximately $1 \ge 10^2$ cfu/100 ml.
- 110 ml of the inoculated test water was poured through the neck of the bottle. Once the entire volume was filtered, the timer was initiated.
- At the designated contact time, 100 ml of the water was filtered through a 0.45 micron filter. Simultaneously, one ml of water was placed in 9 ml of phosphate buffered water with 0.1 N sodium thiosulfate. After filtering the 100 ml, the funnel was ninsed with 100 ml of 0.1N sodium thiosulfate.

The filter was placed on a plate of VRBA and incubated at 35°C for 48 hours. The one red which was neuroalized with 0.1N sodium thiosulfate was plated with VRBA and incubated at 35°C for 48 hours.

UTRALIZATION:

was essential to verify that the neutralization of the active antimicrobial agent as outlined overwas effective. This was accomplished as follows:

One ml of filtered test water was added to 9 ml of 0.1 N sodium thiosulfate phosphate bufferd water.

1 - 100 cfu of K. remigent were added to this test tube and to tan ml of phosphate buffered water without filtered water.

-- Both sets of inoculated water were plate counted with VRBA. Similar plate counts would indicate adequate neutralization.

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RESULTS:

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TABLE 1; NEUTRALIZATION

	K. terrigena A (cfu/ml)	K. terrigna B (cfu/ml)	Average <u>[cfu/ml]</u>
Control	9	16	13
Purified/Neutralized	11	16	14

Summary: Counts between the control sample and the purified and neutralized sample are similar, indicating that the neutralization method employed was effective.

TABLE 2: RECOVERY COUNTS OF INOCULATE DAND PURIFIED WATER

	Contact Time (minutes)	Recovered K. terrig	igena Percent Reduction
기	0	13,300,000	not applicable
_	1.0	18	99_99986%
	2.5	30	99.99977%
D	5.0	39	99.999 70%

Conclusion;

EPA Guide Standard and Protocol for Testing Microbiological Water Purifiers requires a six log reduction of bacterial counts (99.9999%). The data derived from this study indicates a five plus log reduction was achieved by Pres to Pure.

Discussion:

The data indicate that the antibacterial efficacy of this product is not time dependent (at least not after one minute of exposure), as essentially the same results were achieved after 1, 2.5 and 5 minutes of contact time. The surviving organisms represent a plateau in the death/time curve. This plateau may represent a reaction between our test water and the active agent, or of residual bacteria remaining in the nack of the bottle which do not pass through the filter, and therefore do not contact the lodine resin.

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