Germ Pro, Surface Disinfectant (Quick Dry Formula) Efficacy Testing Summaries:

Test Facility: *Consumer Product Testing Co.* Number M95-267.01., 02, .03

1) *Objective:* Determination of relative antimicrobial effectiveness of Germ Pro Surface Disinfectant over a four week period. The method was designed and is intended for obtaining basic information about the relative stability of these products. *P.aueruginosa* A.T.C.C. 9027 was used as the marker microorganism to determine the products' suitability as a surface disinfectant.

***Result:* The lethality of the formulation vs. *P. aueruginosa* was confirmed at 10 and 15 min in Tripticase Soy Broth (TSB) transfers and at maximum dilution of 0.0625% after 0,14, and 28 days. All product dilutions from 2% - 0.0625% tests indicated negative growth**

Test Facility: *Viromed Laboratories Inc.* Number 2669

3) *Objective:* To evaluate the residual self-sanitizing activity of dried product chemical residues on undisturbed hard inanimate surfaces according to test criteria and methods approved by the US EPA for registration of the product. Test organisms used were *Staphylococcus aureus* A.T.C.C. 6538, *Salmonella choleraesuis* A.T.C.C. 10708, and *Pseudomonas aeruginosa* A.T.C.C. 15442.

Several sterile ceramic "test tiles" were treated with a previously prepared broth suspension of the test organism and allowed to dry on the tiles for 40 minutes. The product was then applied by spray to the test tiles subsequently held for 10-15 minutes at room temperature. The tiles were subsequently transferred to a Letheen Broth , processed , and then plated out and incubated at 35-37C in triplicate per organism . The test tiles were subsequently re-challenged at day 14 and day 28 with 0.01 ml of the test organism prior to sampling. A similar procedure was used on several control samples for comparison to the product test samples.

***Results:*The efficacy at time 0 and day 14 was established vs. all organisms tested. For day 28 the percent reduction VS the control samples varied between 28.6% for**

Pseudomonas aeruginosa **to 92.2% for** ***Salmonella choleraesuis*** **with all other organisms tested results falling between these values.**

Test Facility: *MedTech Laboratory* 06/18/2000

4) ***Objective:*** **Efficacy Vs. a standard McFarland suspension of microorganisms (*E.Coli* ATCC# 51299, Salmonella group C1 ATCC#7009, *P. aeruginosa* ATCC#27853 and *S. auereus* ATCC #25923 all prepared in saline solution.**

Test #1 A .5 suspension was transferred to TSA agar with 5% sheep's' blood and plated .25ul of Germ Pro was placed on top of the inoculated agar and incubated for 24 hours at 35 C. Growth was then observed. Test #2 A standardized inoculum of the same microorganisms used in test #1 to a .5 McFarland in 1.8 ml of saline to which 200ul of Germ Pro Surface Disinfectant was added. Each suspension was then transferred to TSA Agar with 5% sheep's' blood and then incubated for 24 hours at 35C. Growth was then observed.

Results: **For both Test #1 and Test #2 no growth was observed on any of the Petri dishes where Germ Pro Surface Disinfectant was placed, indicative of complete inhibition or bactericidal effect.**

Test Facility: ViroMed Laboratories Inc. Number 10524

5) Objective: **The determination of the "Residual Self-Sanitizing Activity" of Germ Pro Surface Disinfectant**

The test organism used for this study was *Salmonella choleraesuis* ATCC#10708. A 0.5 McFarland suspension was prepared in Butterfield's Buffer using a stock culture of the test organism. A "Lawn of Growth" was prepared by adding 0.2 ml of the suspension to agar plates and incubating same for approximately 48 hours at 35-37C. A 3ml aliquot of Butterfield's Buffer was transferred to each agar plate and cell swabbed to suspend the organisms. Suspension was filtered through sterile gauze and diluted to 0.5 McFarland turbidity. Prior to use in the testing procedures, the suspension was Vortex mixed and a 1:50 dilution in Butterfield's Buffer was made.

One sterile glass slide was transferred into each matted (with 9cm filter paper) sterilized glass petri dish as a "carrier". The petri dishes containing the treated carriers were held at room temperature with their lids adjar for 24 hour,72hour,and 7day holding periods. The carriers were allowed to equilibrate to ambient temperature prior to being treated with the Germ Pro Surface Disinfectant which was subsequently sprayed on at a distance of 4-6 inches from the carrier for 3

seconds at which point the carriers were completely wet. A timer was started for the subsequent holding period.

After the specified holding times , 0.01ml of the test organism suspension was added to the designated areas of separate replicates of previously treated carriers at staggered intervals. The test organisms were allowed to contact the Germ Pro Surface Disinfectant residue for 15 minutes at ambient temperature.

Subculture: **Following the 15 minute exposure period, each "carrier" was transferred at identical staggered intervals to 40 ml of Letheen Broth and vortex mixed. The number of survivors from each "carrier" was determined by a standard spread plate method, using TSA with 5% sheep's' blood.**

The colonies present on each plate were counted and recorded and the number of Colony Forming Units (CFU's) present on each "carrier" or suspension was calculated.

Results: **Under conditions of this investigation Germ Pro Surface Disinfectant, quick dry ready to use , is an effective residual sanitizer against *Salmonella choleraesuis* for hard nonporous surfaces after a 24 hour, 72 hour, and 7 day hold with a 15 minute contact time.**

Test Facility: ViroMed Laboratories Inc. Number 1844

Objective: **Determination of the virucidal efficacy of Germ Pro Surface Disinfectant on inanimate environmental surfaces which have been in contact with *Influenza A2* for a 10 minute exposure time.**

The Virus Microorganism used for this test was *Influenza A2, ATCC VR-544, Strain A2 Hong Kong.*

0.2ml of undiluted virus suspension was spread on the bottoms of 100 X 15 mm sterile glass petri dishes. Films were then dried at 21C for 20 minutes and further dried for 30 minutes at 37C. Dried films were then exposed to the ready to use dilution of Germ Pro Surface Disinfectant for 10 minutes. at 21C. The Germ Pro Surface Disinfectant was applied by spraying the surface of the inoculated carrier at a distance of 4-6 inches until the virus film was completely covered. Following the 10 minute exposure the plate was scraped with a plastic cell scraper to re-suspend the plate contents.

Virus-Disinfectant mixture was then separated by filtration through Sephadex LH-20-100 gel prepared in a column and pre-treated with phosphate buffered saline containing 1% albumin then loaded with 2mls of the virus-disinfectant mixture. The filtrate was then tittered by serial dilution for infectivity.

Results: **No virus activity, and no cytotoxicity was evident after the treatment with Germ Pro Surface Disinfectant.**