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Summary of Experiments

Sample: SYNCO CHEMICAL SUPER-KLEEN

PROCEDURE 1

Material and Methods.

Preparation of Microorganisms. Salmonella enterica subspecies arizonae was grown on a Trypticase Soy Agar (TSA) plate and was passed one time. Organisms from this second plate were used to prepare a 0.5 McFarland (approximately 1 x 10^8 CFU*/ml) suspension.

Enumeration of Microorganisms. Serial ten-fold dilutions of the 0.5 McFarland suspension were prepared using sterile saline. Duplicate 1.0 ml samples of the 1 x 10^{-7} and the 1 x 10^{-6} dilution were added to sterile Petri plates. Approximately 10 ml of Trypticase Soy Agar were added to each of the plates and allowed to solidify. Plates were incubated at 37ºC for 24-72 hours and read.

The microorganism suspension was assayed again after 24 hours of incubation.

Sample Preparation. 10 ul of the 0.5 McFarland suspension were added to 9.99 gm of the sample. The sample was mixed. Following initial testing the sample was incubated at room temperature for the duration of the study.

Enumeration of Sample. Two dilutions of the sample were prepared, a 1/50 and a 1/1000. The 1/50 dilution was prepared by mixing 0.2 gm of the sample with 9.8 ml of sterile saline. The 1/1000 dilution was prepared by mixing 0.2 gm of the 1/50 dilution with 3.8 ml of sterile saline. Duplicate 1.0 ml samples of the 1/50 and the 1/1000 dilution were added to sterile Petri plates. After 24 hours of incubation duplicate samples of only the undiluted test mixture were tested. Approximately 10 ml of Trypticase Soy Agar were added to each of the plates and allowed to solidify. Plates were incubated at 37ºC for 24-72 hours and read.

Procedure. Duplicate 1 ml samples of the 1/50 and 1/1000 dilutions were assayed immediately after preparation. After 24 hours, undiluted samples were tested.

**Colony Forming Unit*

Results.

McFarland 0.5 Suspension: Initial assay: 1.5 x 10^8 CFU/ml

Time of Incubation	CFU/ml	Log Inhibition	Percent Inhibition
Initial Sample	0	>5.18	100
1 Day	0	>5.18	100

McFarland 0.5 Suspension: After 24 hours of incubation 1.3 x 10^8 CFU/ml

Discussion and Conclusions.

This experiment was based on USP 51 Antimicrobial Preservative Effectiveness Test, but was substantially modified. One part of a suspension containing 1.5 x 10^8 CFU/ml of Salmonella enterica subspecies arizonae was mixed with 999 parts of the sample. This resulted in a 1000 fold dilution of the microorganism with the test sample (Approximately 1.5 x 10^5 CFU/ml). This sample was quickly assayed and no Colony Forming Units were detected. This indicated that the sample solution had killed or inhibited all of the microorganisms in the approximately 30 minutes it had taken to perform the assay.

The sample was again assayed after 24 hours of incubation. Based on the results of the initial testing instead of assaying the 1/50 dilution and the 1/1000 dilution, duplicate 1.0 ml samples of the test sample were put into sterile Petri dishes and assayed. No organisms were observed.

As a control the microorganism suspension was assayed after 24 hours and found to have essentially the same number of CFUs that it originally had.

The sample not only doesn't support the growth of Salmonellaenterica subspecies arizonae. It appears to be 100% bacteriostatic or bacteriocidal within 30 minutes of testing..

PROCEDURE 2

Material and Methods.

Preparation of Microorganisms. Salmonella enterica subspecies arizonae was grown on a Trypticase Soy Agar (TSA) plate and was passed one time. Organisms from this second plate were used to prepare a 0.5 McFarland (approximately 1 x 10^8 CFU*/ml) suspension.

Enumeration of Microorganisms. Serial ten-fold dilutions of the 0.5 McFarland suspension were prepared using sterile saline. Duplicate 1.0 ml samples of the 1 x 10^{-6} and the 1 x 10^{-7} dilution were added to sterile Petri plates. Approximately 10 ml of Trypticase Soy Agar were added to each of the plates and allowed to solidify. Plates were incubated at 37ºC for 24-72 hours and read.

Sample Preparation. 0.5 gm of the sample were spread over the surface of a sterile Petri plate. 10 ul of the 0.5 McFarland suspension were placed on the surface of the test sample. The sample was incubated for 24 hours at room temperature. Approximately 10 ml of Trypticase Soy Agar was added to the plate and allowed to solidify.

A similar test was run using 0.5 gm of saline as a control.

Plates were incubated at 37ºC for 24-72 hours and read.

**Colony Forming Unit*

Results.

McFarland 0.5 Suspension: 1.5 x 10^8 CFU/ml

Based upon the assay above, the 10 ul sample of the microorganism suspension added to the test sample should have produced approximately 1.5 x 10^6 actual colonies. The result that was obtained is shown in Figure 1. No colonies are observed.

In contrast, as shown in Figure 2, the plate is overgrown. This is consistent with a plate that had been inoculated with over 1 million CFU.

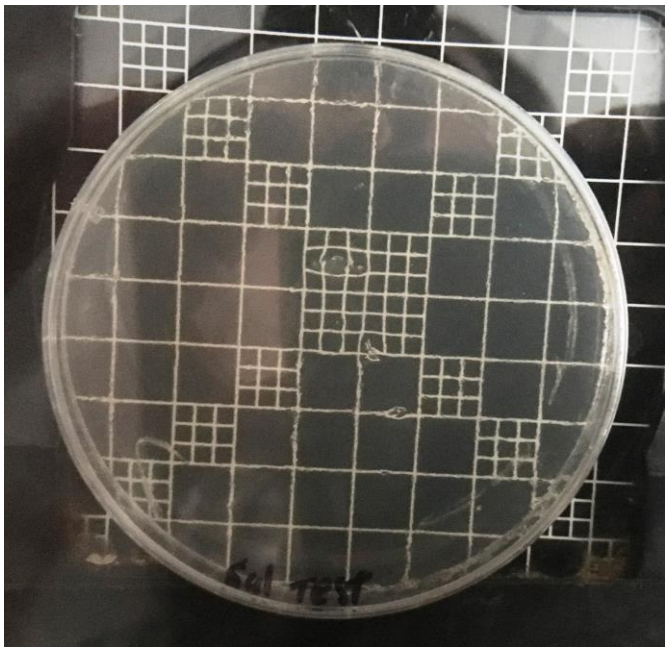

Figure 1. Test Sample

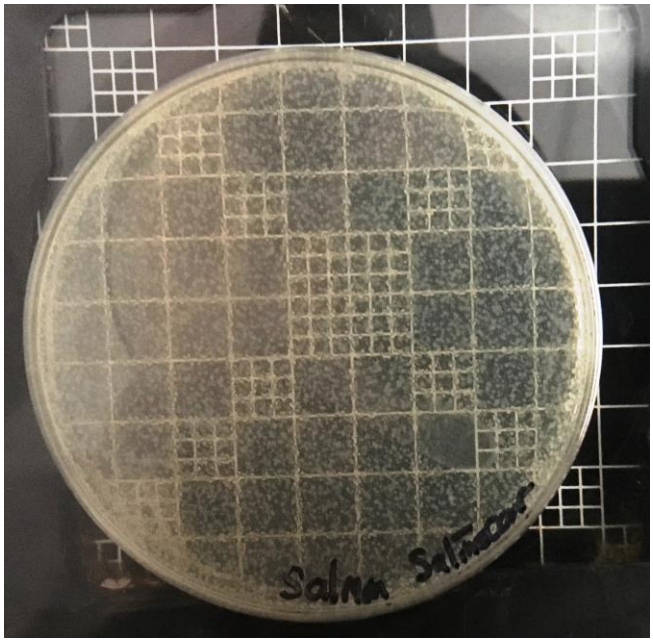

Figure 2 10 ul of 0.5 McFarland Suspension

Discussion and Conclusions.

This experiment was conducted to determine if microorganisms placed on the surface of a sample of the test mixture could survive. As shown above after 24 hours of incubation the number was reduced from approximately 1.5 million down to zero. This result supports the findings in the first set of experiments.

Based on these observations SYNCO CHEMICAL SUPER-KLEEN does not support the growth of Salmonella enterica subspecies arizonae. The results also indicate that in addition to not supporting the growth of these organisms, following contamination the sample reduces the viability of the microorganisms to zero.

Reviewed by: Lorrence H Green

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