Study Title

The Antimicrobial Activity of Stalosan-F in Wet Conditions

MicroChem Laboratory Project ID Numbers

Bacteria or fungus was labeled as 1.0 ml in a sterile plastic petri plate to simulate pooled water or urine. Three petri plates were immediately sprinkled with 0.365g per petri plate per the label directions using a saltshaker and three other petri plates were left untreated as a control. After being mixed and held for an exposure time of 8, 30, 60 minutes, and 8 and 24 hours, the petri plates were tilted on their side, 0.8-0.3 ml was removed and placed into a 9.0 ml tube of neutralizing medium. The tubes were agitated on a vortex mixer for 30 seconds and serial ten-fold dilutions were made as 1.0 ml into 9.0 ml neutralizing medium. One-half (0.5) ml portions of the dilutions were placed onto nutrient agar in petri plates to measure the number of surviving CFU of bacteria or fungus at an exposure time of 20.0 minutes at ambient temperature.

Materials

Stalosan-F

Microbes

Nutrient Broth (NB)
Lot No. 2196531, Exp. 01/07
Difco Laboratories
Detroit, MI

Nutrient Agar (NA)
Lot No. 3183317, Exp. 05/08
Difco Laboratories

Dey Engley Broth (DE)
Lot No. 3141414, Exp. 04/06
Difco Laboratories

Glycine
Lot No. 02613CB, Exp.06/06
Aldrich Chemical Company
Milwaukee, WI

Sterilized Deionized Water (SDIW)

MicroChem Laboratory, Inc.
Euless, TX

Petri Plates, Plastic, Disposable 15x100mm
Fox Scientific
Alvarado, TX

Incubators capable of 35±2°C

Steam Sterilizer, Model STM-E
Market Forge

Glassware:
Test tubes, closures, and support racks
Beakers and flasks a variety of sizes
French square bottles (FSQB)

Pipettes 1.0 ml. 10.00 ml, 50.0 ml, Glass
VWR Scientific
Sugarland, TX

Nichrome Wire Loops
Baxter Diagnostic, Inc.
McGraw Park, IL

Black felt tip marking pen

Scissors

Vortex Mixer
Fisher Scientific

Forceps

Procedures

1. **Grow the Bacterial Cultures:**

The bacterial stock was obtained fresh from the American Type Culture Collection within six months from use in this test. Bacteria or fungus stocks were maintained on nutrient agar slants at 3±2ºC after monthly transfers to fresh nutrient agar slants grown for 48±8 hours at 35±2ºC. From the bacterial stock,

one loopful of bacteria was added to 10.0 ml of nutrient broth and incubated at 35±2ºC for 24±4 hours.

Bacteria was diluted 100 fold as 1.0 ml into 99.0 ml of NB.

2. **Label the 2x2 cm Paper Square Carriers and Petri Plates**

Each sterile petri plate was labeled with 1.0 ml of the culture by using a sterile 1.0 ml glass pipette.

3. **Application of Stalosan-F**

0.365g per petri plate was applied to the test petri plates. The Stalosan-F label directs to use 1.6oz/10.5ft^2. Then covert it to 1 ft^2: 1.6oz = 45.36g/10.5ft^2

$$\frac{45.36\ g}{10.5\ ft^2} = \frac{1\ ft^2}{0.0929\ m^2} = 46.5\ g/m^2$$

Next, to find the area of the petri plate. $A= (\pi)(r^2)$

$$A= 3.14(50)^2 = 7850\ mm^2$$

To find how much Stalosan-F is needed per petri plate.

$$\frac{46.5g}{1\ m^2} = \frac{1m^2}{10^6\ mm^2} = \frac{7850\ mm^2}{1} = 0.365g/\text{petri plate}$$

4. **Measure the Number of Surviving CFU of bacteria or fungus After Exposure to Stalosan F**

After an exposure for the test, the petri plate was slightly tilted on its side. With a 1.0 ml sterile glass pipette, 0.8-0.3 ml was removed from the petri plate and placed into a 9.0 ml tube of DE. The tube was agitated for 30 seconds on a vortex mixer. Serial ten-fold dilutions were made as 1.0 ml into 9.0 ml DE. One-half (0.5) ml samples of dilutions were placed onto nutrient agar (NA) in petri plates. The test tubes and petri plates were incubated for 48±8 hours at 35±2ºC. Colonies were counted and multiplied by dilution factors to determine the number of surviving CFU for each petri plate. The three petri plates were averaged for the test.

As a control, the above procedures were repeated for each test to determine the number of surviving CFU of bacteria or fungus at each exposure time without exposure to Stalosan-F.

5. **Calculation of Percent Kill by Stalosan-F**

C= Control Colony Forming Units T= Test Colony Forming Units

For test 1: Total % Kill = $\frac{C_0 - T_{48}}{C_0} \times 100$

For test 2 and 3: Total % Kill = $\frac{C_0 - T_{20}}{C_0} \times 100$

6. **Validation of Neutralization and Viability**

To validate the neutralization of Stalosan-F, two sterile plastic petri plates were labeled with 1.0 ml of SDIW and 0.365g of Stalosan-F was immediately sprinkled in the petri plate. With a sterile 1.0 ml glass pipette, 0.45 ml was removed from the petri plate and placed into 9.0 ml of neutralizing recovery medium. Each dilution tube was spiked with about 1000 CFU in 1.0 ml and 0.5 ml of each tube was plated onto nutrient agar (NA) in petri plates.

For a comparative number, a ten-fold dilution was made as 1.0 ml into 9.0 ml tube of neutralizing recovery medium. Each dilution tube was spiked with about 1000 CFU in 1.0 ml and 0.5 ml of each dilution tube was plated onto nutrient agar (NA) in petri plates.