

Amazing Concepts LLC

Manufacturer of

NOK-OUT

“The Heavyweight of Odor Eliminators”

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Certificate of Analysis

Product: EPA registered Broad Spectrum Disinfectant #71700-2

Project: Test product effectiveness against **Clostridium difficile** on non-porous surfaces

Testing: SanAir Technologies Laboratory BC-01

Methodology:

All steps were performed aseptically while wearing gloves. The biosafety cabinet (BSC) were emptied of all non-essential items, cleaned, and sterilized with 10% bleach before beginning any work. All tools used were sterilized with alcohol wipes before being placed into the BSC-this include pipettes and forceps. And the slides were manipulated after being sterilized; they must only be handled with sterile forceps.

Sterilization of the test slides:

1. Thirteen plain glass slides were submerged in a 10% bleach solution for 1 hour.
2. The slides were air-dried in the BSC for 30 minutes by standing them on edge on a sterilized surface.
3. Then the slides were soaked in 70% isopropyl alcohol for 5 minutes to remove residual bleach.
4. The slides were air-dried in the BSC.

Inoculating the test slides:

1. The sterilized slides were laid on the surface of the BSC
2. Using a fixed-volume 100ul pipette and a sterile pipette tip, Clostridium difficile bacterial suspension was loaded onto a slide.
 - a) Repeat until 11 of the thirteen slides have been inoculated.
 - b) The 12th slide served as an un-inoculated negative control.
 - c) The 13th slide was treated with product but not inoculated
3. The slides were allowed to lay flat in the BSC until the inoculums dried.

Treating the test slides:

1. Ten of the eleven inoculated slides were placed along with one of the un-inoculated slides side-by-side in the BSC.
2. The bottle of product was held a distance of one foot above the slides.
3. The product was sprayed onto the slides by fully squeezing the handle ten times to ensure that the product evenly covers them and that they were thoroughly wet.
4. The slides were air-dried face-up for 10 minutes

Incubation:

1. Thirteen bottles of thioglycolate broth were prepared. Ten were labeled as experimental bottles, one was labeled as a treated +bacteria, one as a positive control, and one as a negative control.
2. One of the treated inoculated slides was placed into each of the ten experimental bottles.
3. The treated but un-inoculated slide was inserted into the ‘treated+bacteria’ bottle.

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4. 100ul of bacteria from the stock culture was added to the 'treated+bacteria' bottle.
5. The un-treated positive control slide was loaded into the positive control bottle.
6. The un-inoculated negative control slide was placed into the negative control bottle.
7. All of the bottles were sealed and placed into a 35 degree C incubator for 24 hours.

Results:

1. After 24 hours, all the experimental bottles did not display turbidity and were recorded as negative. All Negative bottles were sub-cultured to fresh thioglycolate broth to ensure the negative result and returned to incubator for another 24 hours. The sub-cultured bottle did not display turbidly, which confirmed the negative results of all experimental bottles.
2. The positive control bottle displayed turbidity after 24 hours of incubation. Treated+bacteria bottle also displayed turbidity and was recorded as positive.
3. Each bottle lacking growth was re-inoculated with 100ul of stock culture. They were returned to the incubator for another 24 hours. After 24 hours, 4 out of 10 bottles re-inoculated with stock culture display turbidity. The bottles were returned to incubator for another 24 hours, 8 out of 10 bottles re-inoculated with stock culture were positive after 48 hours incubation. Only two bottles showed the residual biocidal activity in the culture media.

Conclusion:

Product was definitely effective for eradicating *Clostridium difficile* on non-porous Surfaces in 10 minutes.