

**Report submitted to Virox Technologies Inc.
Mississauga, Ontario**

**ASSESSMENT OF THE SANITIZING ACTION OF
VIROX BROAD-SPECTRUM CLEANER AND NO-
RINSE SANITIZER (VCNC)**

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February 2004

A. OBJECTIVE

The main objective of this study was to determine the effectiveness of Virox Broad-Spectrum Cleaner and No-Rinse Sanitizer (VCNC) as a sanitizer at a dilution of 1:128 against *Escherichia coli* and *Staphylococcus aureus* using a suspension test method.

B. MATERIALS AND METHODS

The Product:

One lot of the product was provided for testing in this study. Upon arrival in our laboratory, the bottles were stored at room temperature in a place with restricted access.

The product was tested at a dilution of 1:128.

Soil Load: No soil load was used in this testing.

Neutralizer, Microbial Diluent and Filter Rinse:

Lethen Broth (with 0.1% sodium thiosulfate pentahydrate) was used as the neutralizer. Normal saline was used to make dilutions of the bacterial suspensions and to rinse vials, membrane filters and the filter holder.

Standard Hard Water:

To dilute the product for testing, water with a standard hardness of 200 ppm as CaCO₃ was used as the diluent.

Test Organisms:

The organisms used and their specific strains are given below:

1. *Escherichia coli* (ATCC 25404)
2. *Staphylococcus aureus* (ATCC 6538)

Stock suspensions of the bacteria were prepared by culturing them in tryptic soy broth (TSB; Difco) for 24 hours at 37°C.

C. THE TEST METHODOLOGY

The Suspension Test:

The test was carried out by adding 100 µL of the bacterial suspension without soil load to 900 µL of the test product in a 2 mL capacity cryovial and allowed to sit for the required contact time at room temperature. At the end of the contact time, the reaction mixture was vortexed and transferred into a vial containing 9.0 mL of the neutralizer and vortexed again. Further 1:10 dilutions were done. Both dilutions were separately passed through a membrane filter.

Recovery Media and Detection of Viable Organisms:

For tests using *Escherichia coli* and *Staphylococcus aureus*, the filters were placed on TSA plates, incubated at 37°C, monitored, and the CFU were recorded at 24-hour intervals for a total

of 5 days.

Controls:

Controls were tested by adding 100 µL of bacterial suspension into 900µL saline instead of the disinfectant.

D. PRODUCT PERFORMANCE CRITERIA

The numbers of test repeats in the bactericidal were 5. The test also included three control repeats. The results are reported as log₁₀ reductions in viability in reference to the control.

For a product to be considered bactericidal it was expected to reduce the viability titre of each test organism by at least 3 log₁₀ under the conditions of this test.

E. RESULTS

The activity of VCNC against *E. coli* (ATCC 25404): Table 1 summarizes the results of the suspension test. The product was able to bring about a >5 log₁₀ reduction in the viability titre of *E. coli* in a contact time of 30 seconds at room temperature.

Table 1: The activity of a 1:128 dilution of VCNC against *E. coli* (ATCC 25404)

| Date of Expt. | Dilution | Lot No. | # of Repeats | Contact Time | CFU/control | CFU/test | Log ₁₀ Reduction |
|---------------|----------|---------|--------------|--------------|------------------------|----------|-----------------------------|
| 20/01/04 | 1:128 | 3597 | 5 | 30 sec | 1.07 X 10 ⁵ | 0 | 5.03 |

Table 2 summarizes the activity of VCNC against *S. aureus*. The product was able to bring about a >5log₁₀ reduction in the viability titre of *S. aureus* in a contact time of 30 seconds at room temperature.

Table 1: The activity of a 1:128 dilution of VCNC against *S. aureus*

| Date of Expt. | Dilution | Lot No. | # of Repeats | Contact Time | CFU/control | CFU/test | Log ₁₀ Reduction |
|---------------|----------|---------|--------------|--------------|------------------------|----------|-----------------------------|
| 22/01/04 | 1:128 | 3597 | 5 | 30 sec | 1.47 X 10 ⁶ | 0 | 6.15 |

F. CONCLUDING REMARKS

VCNC when diluted 128-fold, was able to reduce viability titre of *E. coli* (ATCC 25404) and *S. aureus* by a >4 log₁₀ and 6 log₁₀, respectively, with a contact time of 30 seconds at room temperature in a suspension test. This preliminary testing was carried out on one lot of the product. Additional lots of product should be tested against these organisms with a neutralization assay; this would be necessary to generate full support data required for any submission for product registration.