

Excerpts of Laboratory Test Results of Vital Oxide
Batch # 106-105-1 and Batch #901120 on Swine
Influenza A (H1N1) Virus

STUDY TITLE

Virucidal Efficacy of a Disinfectant for Use on
Inanimate Environmental Surfaces

Virus: Swine Influenza A (H1N1) virus

PRODUCT IDENTITY

Vital Oxide
Batch # 106-105-1 and Batch # 901120

PURPOSE

The purpose of this study is to evaluate the virucidal efficacy of a test substance against Swine Influenza A (H1N1) virus according to test criteria and methods approved by the U.S. Environmental Protection Agency for registration of a product as a virucide.

VIRUS

The A/Swine/Iowa/15/30 strain of Swine Influenza A (H1N1) virus to be used for this study was obtained from the American Type Culture Collection, Manassas, VA (ATCC VR-333). Stock virus is prepared by collecting the supernatant culture fluid from 75-100% infected culture cells. The cells are disrupted and cell debris removed by centrifugation. The supernatant is removed, aliquoted, and the high titer stock virus may be stored at ≤ -70°C until the day of use. On the day of use an aliquot is removed, thawed and refrigerated until use in the assay. **Note:** If the Sponsor requests an organic soil load challenge, fetal bovine serum (FBS) will be incorporated into the stock virus aliquot. The percent FBS contained in the stock virus aliquot will be adjusted to yield the percent soil load requested.

TABLE 1: Virus Control and Test Results

Effects of Vital Oxide (Batch # 106-105-1 and Batch # 901120) Following a Five Minute Exposure to Swine Influenza A (H1N1) Virus Dried on an Inanimate Surface

Dilution	Dried Virus Control (GROUP A)	Swine Influenza A (H1N1) virus + Batch # 106-105-1 (GROUP B)	Swine Influenza A (H1N1) virus + Batch # 901120 (GROUP B)
Cell Control	0 0 0 0	0 0 0 0	0 0 0 0
10 ⁻¹	++++	TTTT	TTTT
10 ⁻²	++++	0 0 0 0	0 0 0 0
10 ⁻³	++++	0 0 0 0	0 0 0 0
10 ⁻⁴	++++	0 0 0 0	0 0 0 0
10 ⁻⁵	++00	0 0 0 0	0 0 0 0
10 ⁻⁶	0 0 0 0	0 0 0 0	0 0 0 0
TCID ₅₀ /0.1 mL	10 ^{5.0}	≤10 ^{1.5}	≤10 ^{1.5}

(+) = Positive for the presence of test virus

(0) = No test virus recovered and/or no cytotoxicity present

(T) = Cytotoxicity present

STUDY RESULTS

Results of tests with two batches of Vital Oxide (Batch # 106-105-1 and Batch # 901120), ready to use and applied using a trigger spray, exposed to Swine Influenza A (H1N1) virus in the presence of a 5% fetal bovine serum soil load at room temperature (20.0°C) for five minutes are shown in Tables 1-3. All cell controls were negative for test virus infectivity. The titer of the dried virus control was 5.0 log₁₀. Following exposure, test virus infectivity was not detected in the virus-test substance mixture for either batch at any dilution tested (≤1.5 log₁₀). Test substance cytotoxicity was observed in both batches at 1.5 log₁₀. The neutralization control (non-virucidal level of the test substance) indicates that the test substance was neutralized at ≤1.5 log₁₀ for both batches. Taking the cytotoxicity and neutralization control results into consideration, the reduction in viral titer was ≥3.5 log₁₀ for both batches.

SUMMARY OF RESULTS

Test Substance: Vital Oxide, Batch # 106-105-1 and Batch # 901120

Dilution: Ready to use, applied using a trigger spray

Virus: Swine Influenza A (H1N1) virus, ATCC VR-333, Strain A/Swine/Iowa/15/30

Exposure Time: Five minutes

Exposure Temperature: Room temperature (20.0°C)

Organic Soil Load: 5% fetal bovine serum

Efficacy Result: Two batches of Vital Oxide (Batch # 106-105-1 and Batch # 901120) met the test criteria specified in the study protocol. The results indicate **complete inactivation** of Swine Influenza A (H1N1) virus under these test conditions as required by the U.S. EPA for claims of virucidal activity.