## OBJECTIVE

The objective of this study was to evaluate the virucidal efficacy of a test substance for registration of a product as a virucide. The test procedure was to simulate the way in which the product is intended to be used. This method is in compliance with the requirements of and may be submitted to the U.S. Environmental Protection Agency (EPA).

## SUMMARY OF RESULTS

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|---|---|
| Test Substance: | AME Antimicrobial, Lot 020420 and Lot 040420 |
| Dilution: | Ready to use (RTU), applied as a trigger spray |
| Virus: | Human Coronavirus, ATCC VR-740, Strain 229E |
| Exposure Time: | 5 minutes |
| Exposure Temperature: | Room temperature (20.0°C) |
| Exposure Humidity: | 50% |
| Organic Soil Load: | 1% Fetal Bovine Serum |
| Efficacy Result: | Two lots of AME Antimicrobial (Lot 020420 and Lot 040420) met the performance requirements specified in the study protocol. The results indicate **a ≥3 $\log_{10}$ reduction in titer** of Human Coronavirus under these test conditions as required by the U.S. EPA. |



## TEST SYSTEM

1. Virus

The 229E strain of Human Coronavirus used for this study was obtained from the American Type Culture Collection, Manassas, VA (ATCC VR-740). The stock virus was prepared by collecting the supernatant culture fluid from 75-100% infected culture cells. The cells were disrupted and cell debris removed by centrifugation at approximately 2000 RPM for five minutes at approximately 4°C. The supernatant was removed, aliquoted, and the high titer stock virus was stored at ≤-70°C until the day of use. On the day of use, an aliquot of stock virus (Accuratus Lab Services Lot HCV-85) was removed, thawed and maintained at a refrigerated temperature until used in the assay. The stock virus culture was adjusted to contain 1% Fetal Bovine Serum as the organic soil load. The stock virus tested demonstrated cytopathic effects (CPE) typical of Coronavirus on WI-38 (human lung) cells.