

The Anti-Viral Efficacy of a New Super-Oxidized Solution

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Objective

To perform an *in-vitro* evaluation of the efficacy of Super-Oxidized Solution, SOS (Microcyn™) against a representative range of viruses.

Background

A new stable, pH neutral, super-oxidized solution (SOS), Microcyn™, has been shown to possess *in vitro* antibacterial, sporocidal and antiviral activities. For example, following a five (5) minute exposure to Microcyn™, HIV-1 load on hard surfaces (glass) was reduced by a log₁₀ factor > 3. A similar inactivation effect was found with adenoviral vectors type five 5 (Landa *et.al.*, JHI 2005). This study was conducted to evaluate the inactivation of various enveloped and non-enveloped viruses exposed to Microcyn™ under simulated laboratory in-use conditions with viral suspensions. Challenge viruses included poliovirus-1, rhinovirus-1A, respiratory syncytial virus (RSV), herpes simplex virus-1 (HSV-1), herpes simplex virus-2 (HSV-2), influenza-A H1N1, influenza-A H3N1, West Nile virus (WNV), and feline calicivirus (FCV) [as a surrogate for Norwalk Virus].

Materials & Methods

Target cells selected for virus type were infected with either control- or SOS-treated viruses. Control virus (10⁸-10¹⁰ plaque-forming units [pfu]) was exposed to 1 ml of saline for ten (10) minutes; then diluted in one (1) ml of 10% serum supplemented culture medium. The virus-SOS mixtures were prepared with control-matched concentrations of the viruses exposed to 1 ml of SOS for one (1) and five (5) minutes; then diluted in 1ml supplemented medium for SOS inactivation. Cells in forty-eight (48) well culture plates were infected with 300 µl control or treated viruses/well, then incubated for one hr at 36°C, 5%CO₂. At the end of the exposure period, all wells received additional medium. At 24-48 hrs post infection cells were examined for cytopathic effects.

Results

For poliovirus-1, a log₁₀ reduction factor of at least four (4) was achieved after one (1) minute exposure to Microcyn™ vs. the control. Rhinovirus 1A was reduced by eight (8) log₁₀ after one (1) minute exposure to Microcyn™. A four (4) log₁₀ reduction was achieved for RSV. For both HSV-1 and HSV-2, log₁₀ reductions of six (6) were achieved following exposure for one (1) minute. For both strains of influenza-A virus, there was at least a seven (7) log₁₀ reduction following exposure to Microcyn™ for one (1) minute. WNV was reduced by seven (7) log₁₀ after one (1) minute; and by eight (8) log₁₀ after five (5) minutes. For FCV, a five (5) log₁₀ reduction was achieved after one (1) minute, increasing to a six (6) log₁₀ reduction after five (5) minutes. All of these log-reduction values were at the limit of sensitivity for each of the specific assays.

Table 1: Microcyn Viral Inactivation Results (TCID₅₀)

Virus	Envelope	Control Plate	1 min exposure	5 min exposure	Reduction of Infectivity
FCV 2280	No	>10 ⁻⁹	10 ⁻⁴	10 ⁻³	>10 ⁵
Flu A H1N1	Yes	>10 ⁻¹⁰	<10 ⁻²	<10 ⁻²	>10 ⁷
Flu A H3N1	Yes	10 ⁻¹⁰	<10 ⁻²	<10 ⁻²	>10 ⁷
HSV1	Yes	>10 ⁻¹⁰	<10 ⁻²	10 ⁻²	10 ⁶
HSV2	Yes	>10 ⁻⁹	10 ⁻³	10 ⁻³	>10 ⁶
Polio 1	No	>10 ⁻¹⁰	10 ⁻⁶	10 ⁻⁶	>10 ⁴
Rhino 1A	No	>10 ⁻¹⁰	>10 ⁻²	<10 ⁻²	>10 ⁸
RSV	Yes	>10 ⁻¹⁰	10 ⁻⁶	10 ⁻⁶	>10 ⁴
WNV	Yes	>10 ⁻¹⁰	10 ⁻³	<10 ⁻²	>10 ⁷

Conclusion

Taken together, the results demonstrate that the load of enveloped and non-enveloped viruses exposed to Microcyn™ for at least one (1) minute is reduced by a log₁₀ factor ≥ 5. Under these suspension conditions, a complete inactivation was achieved after five (5) minutes of exposure to this SOS product. In light of the significant anti-viral activity of Microcyn™, clinical applications should be investigated.

Example 1: WNV Inactivation Results (TCID₅₀)

West Nile Control Plate. TCID ₅₀ > 10 ¹⁰						
Dilutions	A	B	C	D	E	F
Cell Control	0	0	0	0	0	0
10 ⁻⁴	4+	4+	4+	4+	4+	4+
10 ⁻⁵	4+	4+	4+	4+	4+	4+
10 ⁻⁶	4+	4+	4+	4+	4+	4+
10 ⁻⁷	3-4+	3-4+	3-4+	3-4+	3-4+	3-4+
10 ⁻⁸	3+	3+	3+	3+	3+	3+
10 ⁻⁹	3+	3+	3+	3+	3+	3+
10 ⁻¹⁰	3+	3+	3+	3+	3+	3+

West Nile One Minute Plate. TCID ₅₀ = 10 ⁻³						
Dilutions	A	B	C	D	E	F
Cell Control	0	0	0	0	0	0
10 ⁻²	+	+	+	+	+	+
10 ⁻³	0	+/-	+/-	0	0	+
10 ⁻⁴	0	0	0	0	0	0
10 ⁻⁵	0	0	0	0	0	0
10 ⁻⁶	0	0	0	0	0	0
10 ⁻⁷	0	0	0	0	0	0
10 ⁻⁸	0	0	0	0	0	0

West Nile 5 Minute Plate. TCID ₅₀ < 10 ⁻²						
Dilutions	A	B	C	D	E	F
Cell Control	0	0	0	0	0	0
10 ⁻²	0	0	0	+/-	0	0
10 ⁻³	0	0	0	0	0	0
10 ⁻⁴	0	0	0	0	0	0
10 ⁻⁵	0	0	0	0	0	0
10 ⁻⁶	0	0	0	0	0	0
10 ⁻⁷	0	0	0	0	0	0
10 ⁻⁸	0	0	0	0	0	0

Legend:

0 = No Visible CPE

+/- = Initial signs of CPE developing, but significantly less than 25%

++ = Up to 25% of cells exhibit CPE

2+ = Up to 50% of cells exhibit CPE

3+ = Up to 75% of cells exhibit CPE

4+ = Up to 100% of cells exhibit CPE