



Skanda Life Sciences Pvt. Ltd

DSIR recognized & CPCSEA Approved

Sy No. 47, #10, 11, 12, Sri Shaila Bramara Complex, Sriganada Kaval, Nagarbhavi, Bangalore – 560091



TEST REPORT

Virucidal Activity of Test Sample Zyvex Advanced Oral Spray against H1N1 virus



Work carried out at

Skanda Life Sciences Pvt Ltd,

DSIR recognized R & D centre, Bangalore

Skanda Life Sciences Pvt Ltd.,

10, 11, 12, Sri Shaila Bramara complex, Chandana Layout,

Sriganada Kaval, Nagarbhavi, Bangalore – 560091, Email: info@skandalifesciences.com

R&D centre:

Sy No. 47, No.10-11,
Sree Shaila Bramara Complex,
Kebballahal Main road,
Sunkadakatte,, Bangalore-560091

Animal Facility:

Plot 67-P8, Phase II, Sector II,
KIADB industrial Area, Bidadi,
(Adjacent to BOSCH)
Ramanagara - 562109

Contacts:

Office : +91-9945525767 / 8217430509
Mobile: +91 9738375537/ 9632280882
Email: bd@skandalifesciences.com
Web : www.skandalifesciences.com

Client Details:**Antonio Bianchi**

General Director

Crab Sinergy Srl

Email: crabsinergy@gmail.com

Report No.:

SLSPL/2021/CB/11/040/01

Date of Report issue:

16/12/2021

Date of study initiation:

03/12/2021

Date of Completion:

13/12/2021

Date of Sample receipt:

22/11/2021

Test Lab:

Skanda Life Sciences Pvt. Ltd

No.10-11, Sree Shaila Bramara Complex,

Kebballahal Main road,

Sunkadakatte,, Bangalore-560091

Sample Particulars:

Oral Spray

Sample condition upon receipt

Good and Undamaged

Sample Qty:

30ml

Sample Label & Batch No:

Zyvox Advanced Oral Spray, LOT No: ZOS 003, Exp Date: 05/2024

Nature of sample:

Liquid

Sample preparation:

Ready to use (RTU) – 100%

Test virus:

Influenza A Virus (H1N1), ATCC®VR-1469™, ATCC, USA

Cell line:

Madin-Darby canine kidney, MDCK, ATCC®CCL -34™, ATCC, USA

Method:

American Society for Testing and Materials (ASTM) - E1053

RESULT SUMMARY

Test Virus	Test sample	Sample Concentration	Contact time (mins)	Log reduction of virus titre	Results in % reduction	Efficacy Criteria >4 Log reduction
<i>H1N1</i>	Zyvox Advanced Oral Spray LOT No: ZOS 003	100%	1	3.33	99.9%	Pass
			2	4.67	99.99%	
			5	6.33	99.9999%	
			10	7.33	99.9999%	
			60	7.33	99.9999%	

Conclusion:

- Zyvox Advanced Oral Spray, LOT No: ZOS 003 shows a log reduction of 7.33logs at a concentration of 100% as tested after 60 minutes against *H1N1* virus.



Tested By
Sagar S
Scientist



Authorized Signatory
Dr. Anand S
R and D Head

Protocol:

Cell culture and maintenance

MDCK cell line was procured from ATCC, stock cells was cultured in EMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) in a humidified atmosphere of 5% CO₂ at 37°C until confluent. The cell was dissociated with cell dissociating solution (0.2 % trypsin, 0.02 % EDTA, 0.05 % glucose in PBS). The viability of the cells are checked and centrifuged. Further, 50,000 cells/well were seeded in a 24 well plate and incubated for 24hrs at 37°C, 5% CO₂ incubator.

Virus growth medium

EMEM supplemented with 1mM HEPES, 1µg/ml of TPCK trypsin and 1% antibiotic

Virus neutralization assay procedure

1. A high titre virus stock suspension with a minimum infectivity titre of 10⁸ TCID₅₀ units per ml was removed from cryopreservation and thawed.
2. Test substance was used as such. The required number of sterile dishes were pre-labelled and readily kept.
3. **E1053:** For the virucidal activity assay of Spray, one part of the thawed virus stock suspension was inoculum is spread over the entire surface of a glass Petri dish and allowed to dry. To the dried surface, 0.9ml of test substance was added. The dishes were incubated for the pre-determined time points at room temperature (24°C).
4. At the end of each incubation time, the contents in the dishes were thoroughly mixed and the test substance in the virus-test suspension (0.1ml) was neutralized by performing a 10-fold serial dilution into D/E Neutralizing Broth (0.9ml). The subsequent dilutions were made using virus growth medium up to 10⁻⁸, the contents from each tube was then added onto MDCK host cell monolayer grown in 24-well plates up to sub-confluent level.
5. For the cytotoxicity control, D/E Broth was diluted 10-fold using 9 parts of test solution by pipetting 0.1ml of D/E Broth into a tube containing 0.9 ml of the test substance yielding 10⁻¹ dilution. A second 10-fold dilution was made by adding 0.1 ml of the test substance-D/E mixture into 0.9 ml of D/E Neutralizing Broth constituting the 10⁻² dilution. Further 10-fold dilutions were performed using virus growth medium up to 10⁻⁸, the contents from each tube was then added onto MDCK host cell monolayer grown in 24-well plates up to sub-confluent level.

6. For the neutralization control, 0.1ml of D/E Broth was pipetted into a tube containing nine parts 0.9 ml of the test solution (10^{-1} dilution). Subsequent dilutions up to 10^{-8} were similarly carried out as followed in cytotoxicity control preparations. Following inoculation of the cell culture wells with the neutralized test substance; $\sim 10^2$ TCID₅₀ viral loads were plated onto each cell culture well for all plated dilutions (10^{-2} to 10^{-8}).
7. The virus control titre was performed by adding 0.1ml of virus stock to 0.9 ml of the test/assay medium (virus growth medium). Ten-fold serial dilutions followed in the test/assay medium through 10^{-8} , with each dilution plated in 24-well plate.
8. Following inoculation of the host cells, the multi-well plates were incubated at 37°C with 5% CO₂ for 30 minutes on an orbital rotator to facilitate virus adsorption.
9. One-ml of virus growth medium was then added to each well of the host cell trays, and the MDCK host cell-H1N1 assay plates were incubated for until CPE is observed (7-9 days) at 37°C in a 5% CO₂ atmosphere.
10. Virus control, virus test, cytotoxicity/neutralization controls, and sterility controls were assayed concurrently.

Virus reductions were calculated according to the Spearman-Kärber Method, and reported

Results:

Key: + = Virus recovered; - = Virus not recovered and/or no cytotoxicity observed; T = Toxicity observed

Table 1: Virus suspension time-kill results of Test Samples

Dilution	Virus control			Virus Test Suspension														
				Zyvex Advanced Oral Spray, LOT No: ZOS 003														
				1 min			2 min			5 min			10 min			60 min		
10^{-1}	+	+	+	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
10^{-2}	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
10^{-3}	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
10^{-4}	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
10^{-5}	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10^{-6}	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10^{-7}	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10^{-8}	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	$10^{7.83}$ TCID ₅₀ / 0.1ml			$10^{4.50}$ TCID ₅₀ / 0.1ml			$10^{3.17}$ TCID ₅₀ / 0.1ml			$10^{1.50}$ TCID ₅₀ / 0.1ml			$10^{0.5}$ TCID ₅₀ / 0.1ml			$10^{0.5}$ TCID ₅₀ / 0.1ml		

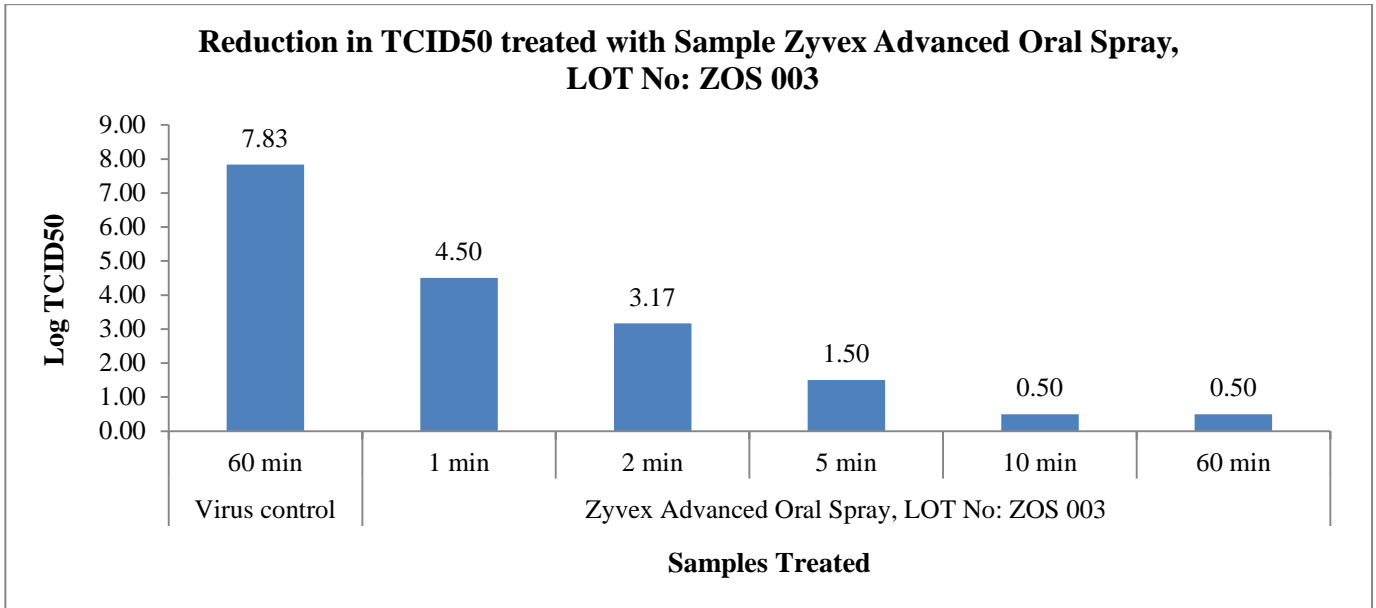


Fig 1: Virus TCID₅₀ reduction results of Sample Zyxex Advanced Oral Spray, LOT No: ZOS 003

Table 2: Log₁₀ Reduction Values for H1N1 testing

Sl. No.	Contact time (mins)	Samples	Sample Conc	Log ₁₀ TCID ₅₀ / 0.1ml	Log ₁₀ Reduction
1	60	Virus Control	-	7.83	0.00
2	1	Zyxex Advanced Oral Spray, LOT No: ZOS 003	RTU	4.50	3.33
	2			3.17	4.67
	5			1.50	6.33
	10			0.50	7.33
	60			0.50	7.33

Table 3: Cytotoxicity and Neutralization validation control data for the given samples

Dilution	Cytotoxicity			Neutralization (Low Titre H1N1)		
	10 ⁻¹	T	T	T	T	T
10 ⁻²	-	-	-	+	+	+
10 ⁻³	-	-	-	+	+	+
10 ⁻⁴	-	-	-	+	+	+
10 ⁻⁵	-	-	-	+	+	+
10 ⁻⁶	-	-	-	+	+	+
10 ⁻⁷	-	-	-	+	+	+
10 ⁻⁸	-	-	-	+	+	+
10 ^{1.50} TCCD ₅₀ / 0.1 ml						
*TCCD ₅₀ - Tissue culture cytotoxic dose value						

-----END OF REPORT-----