DEVELOPMENT OF RAPID ASSAY FOR SCREENING COMPOUNDS
FOR ANTIVIRAL ACTIVITY AGAINST RNA VIRUSES

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A rapid assay for screening samples from marine sources has been developed with a coronavirus model using mouse hepatitis virus, strain A59. Marine samples tested for antiviral activity included species of sponges, gorgonians, algae, and other marine life. These were collected from different environments in Bahamian waters by snorkel, scuba, and submersibles at depths ranging from 2 to 2700 feet.

Eight hundred forty-nine samples were tested against two RNA viruses, A59 and VSV (vesicular stomatitis virus). VSV samples were tested in CV-1 cells, a continuous passage line of green monkey kidney. Antiviral activity against VSV was measured in a plaque reduction assay and a neutral red indicator for viable cells. A59 was propagated in NCTC 1469 cell cultures and antiviral activity was measured by a reduction in cell fusion and cytopathic effects. Cell fusion observed in NCTC 1469 cell cultures infected with strain A59 was observed microscopically in 12 hours and when stained with methylene blue dye the syncytia were visible to the eye as dark blue foci on the fixed cell sheet. Twenty-four hours after infection cell fusion and cytopathic effects were extensive and the assays could be read both macroscopically and microscopically.

Table 1. Toxicity and Antiviral Activity Defined for VSV Replicating in CV-1 Cells and A59 in NCTC 1469 Cells

VIRUS-CELL SYSTEM	CYTOTOXIC READINGS	DEFINITION	ANTIVIRAL READINGS	DEFINITION
A59-NCTC1469	0	Nontoxic	+++	Active Sample
	25	% Cell Kill	++	Partial Activity
	50	% Cell Kill	+	Partial Activity
	75	% Cell Kill	+/-	Marginal Activity
	100	% Cell Kill	-	No Detectable Activity
VSV-CV-1	0	Nontoxic	+++	Active Sample
	8	MM Zone-Cell Kill	++	Partial Activity
	10	MM Zone-Cell Kill	+	Partial Activity
	12	MM Zone-Cell Kill	+/-	No Detectable Activity
	14	MM Zone-Cell Kill	-	Marginal Activity
	16	100% Cell Kill		

Table 2. Antiviral Activity of Marine Samples Against A59 and VSV

VIRUS	TOTAL SAMPLES	TOXICITY					ANTIVIRAL ACTIVITY*				
A59	849	0	25	50	75	100	+++	++	+	+/-	-
		678	21	21	30	99	65	70	31	41	520
		%	80	2	2	3	12	8	8	4	5
VSV	849	0	8-10	12	14	16	+++	++	+	+/-	-
		527	116	38	63	105	18	33	30	45	614
		%	62	14	4	7	12	2	4	4	5

*122 samples were toxic for NCTC 1469 cells and 109 for CV-1 cells.

Of the 65 samples which produced a 3+ antiviral activity against A59, 7 were collected by snorkel (20 feet), 13 by scuba (20 to 90 feet), and 45 by submersibles (100 to 2700 feet). Activities were identified in 24 genera of sponges and 3 other life forms.

Eight percent of the samples were identified as active against A59 at a 3+ level compared to 2% for VSV. If a sample was identified as active against VSV and not A59, this samples was also active against HSV-1 or highly cytotoxic. Eight samples which showed a 3+ activity against VSV were active against A59 at the same level.

A59 results are obtained in 24 hours compared to 72 hours for the VSV assay. The A59 model expedites the work of the chemist for following the activity of compounds purified from crude extracts. It is a strong indicator system for activity against RNA viruses and can be used as an effective screening model.