

Rapid Method to Determine Labeling Specificity of Radioactive Enteroviruses

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Received for publication 29 June 1972

The specificity of labeling enteroviruses with ³²P-labeled NaH₂PO₄ or ¹⁴C-leucine can be determined by comparing the percent adsorption of infective particles and radioactivity to membrane (Millipore) filters.

The procedures most commonly used to determine the purity of radioisotope-labeled enteroviruses have been comparisons of radioactivity and infectivity peaks after density gradient centrifugation (4, 5, 7) or after column chromatography (3, 6). Another method used is to compare uptake of radioactivity and infectivity by susceptible tissue cultures (5). These methods appear to be reliable, but are also time consuming. For this reason, we developed a rapid method of determining the specificity of labeling by use of a membrane (Millipore) technique.

Tissue cultures used for propagation and quantitation of virus stocks were primary rhesus monkey kidney monolayers. The preparation of these cultures and their use for virus assay by the plaque technique in our laboratory have been described (2). The viruses used in this study and the procedures for labeling them with ³²P-labeled NaH₂PO₄ or ¹⁴C-leucine were previously described (Cliver and Herrmann, *Water Res.* 6, *in press*). Purification of labeled virus was done by passage through a diethylaminoethyl-Sephadex A-25 (Pharmacia, Uppsala, Sweden) column (3). All counting of radioactive samples was done with a gas-flow, Mylar window counter.

The membrane adsorption technique used to determine labeling specificity was based on the finding that enteroviruses adsorb to cellulose nitrate membranes whose pore sizes greatly exceed the virus particles' diameter (1). However, if low concentrations of serum (1% or more) were added to the virus suspension, no adsorption took place. To utilize this phenomenon for present purposes, a portion of the Sephadex A-25 column eluate that contained both peak virus plaque-forming units (PFU)

and peak radioactivity was added to phosphate-buffered saline (PBS), pH 7.2, or to PBS with 5% (v/v) calf serum (PBS-Cf_s) added (0.1 ml of virus to 5 ml of diluent). This was filtered through a membrane filter (220 nm porosity, Millipore Corp., Bedford, Mass.). The filtrate was assayed for radioactivity and virus content; the filter, when assayed, was tested for radioactivity content only.

The results of a representative experiment are shown in Table 1. With ³²P-labeled coxsackievirus type A9 (CA9) suspended in PBS, it was found that most of the radioactivity was retained on the membrane filter, whereas ³²P-labeled CA9 in PBS-Cf_s readily passed into the filtrate. The passage or retention of radioactivity through the membranes correlated well with the infectivity data. Less than 1 PFU of CA9 in PBS passed the filter out of an input 3.3 × 10⁶ PFU/ml, whereas 100% of CA9 in PBS-Cf_s passed through the filter. Because a maximum of 5% of the total label in virus suspended in PBS would not adsorb to the membrane, the maximum nonviral label (labeled contaminants or virus breakdown products) was taken to be 5% also. In adsorption experiments with ¹⁴C-leucine-labeled enteroviruses suspended in PBS, similar results were obtained (Table 2). However, because the membrane filters blocked some of the ¹⁴C-activity, only the filtrates were assayed. As a control for possible adsorption of nonviral cellular products, labeled noninfected cell debris (not passed through Sephadex A-25) was filtered in the same manner as that used for virus samples. It was found that more than 88% of the total radioactivity passed the filters.

For both ¹⁴C- and ³²P-labeled preparations, it was found that fractions taken from column effluents after the virus infectivity peaks did not adsorb to membrane filters. Also, solutions

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TABLE 1. Membrane filter adsorption of ³²P-labeled CA9

Sample	PBS diluent		PBS-Cf ₈ diluent	
	Counts/min	PFU/ml	Counts/min	PFU/ml
Starting material	5,435	3.3 × 10 ⁶	5,030	4.0 × 10 ⁶
Filter membrane	5,179		143	
Filtrate	270	<1	4,810	4.7 × 10 ⁶

TABLE 2. Adsorption of ¹⁴C-leucine-labeled enteroviruses to membrane filters

Virus type	Counts/min per sample		Per cent counts/min passed
	Starting material	Filtrate	
Coxsackievirus A7	12,970	650	5.0
A9	9,470	380	4.0
B2	6,040	290	4.8
Echovirus 6	5,320	310	5.8
Poliovirus 1	8,250	350	4.2

of stock ¹⁴C-leucine and ³²P-labeled NaH₂PO₄ suspended in PBS were found not to adsorb to the filters. Based on these findings, routine assays of labeled enterovirus preparations can

be made by measuring membrane filter adsorption of radioactive content alone, without the need to wait for the results of infectivity tests.

This research was supported by the College of Agriculture and Life Sciences and the Biomedical Sciences Committee of the Graduate School, University of Wisconsin, Madison; by the John A. Hartford Foundation, Inc.; and by the World Health Organization.

LITERATURE CITED

1. Cliver, D. O. 1967. Enterovirus detection by membrane chromatography p. 139-141. *In* G. Berg (ed.), Transmission of viruses by the water route. Interscience Publishers, New York.
2. Cliver, D. O., and R. M. Herrmann. 1969. Economical tissue culture technique. *Health Lab. Sci.* 6:5-17.
3. Giron, D. J. 1966. An improved method for the radioisotopic labeling of poliovirus and vaccinia virus. *SAM-TR-66-29*, USAF School of Aerospace Medicine, Brooks Air Force Base, Texas.
4. Henry, C., and J. S. Younger. 1963. Studies on the structure and replication of the nucleic acid of poliovirus. *Virology* 21:162-173.
5. Joklik, W. K., and J. E. Darnell, Jr. 1961. The adsorption and early fate of purified poliovirus in HeLa cells. *Virology* 13:439-447.
6. Martin, S. J., M. D. Johnston, and J. B. Clements. 1970. Purification and characterization of bovine enteroviruses. *J. Gen. Virol.* 7:103-113.
7. Sobsey, M. D., M. E. Lavigne, and R. C. Cooper. 1972. Preparation of poliovirus labeled with phosphorus-33. *Appl. Microbiol.* 23:623-627.