Use of Membrane Filters to Facilitate the Recovery of Virus from Aqueous Suspensions[1]

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ABSTRACT

METCALF, T. G. (University of New Hampshire, Durham). Use of membrane filters to facilitate the recovery of virus from aqueous suspensions. Appl. Microbiol. **9**:376–379. 1961.—Influenza virus was recovered from aqueous suspensions by means of membrane filters. Separation of virus from bacterial mixtures was achieved and virus was recovered free from bacteria. Technics developed for this application of the use of membrane filters were presented. These technics were extended to the successful isolation of Asian influenza virus from clinical specimens made available for use in the study.

The use of membrane filters for the collection and enumeration of bacteria is well established. The essential apparatus and procedures have been described by Goetz and Tsuneishi (1951) and Clark et al. (1951).

One of the more significant aspects of membrane filter usage has been the collection of all of the bacteria present in a test sample (Kabler and Clark, 1952). This has increased the likelihood of recognizing the presence of small numbers of bacteria which might otherwise fail to be detected (Shipe and Cameron, 1954).

The successful application of the use of membrane filters to the detection of bacteria suggested that a similar advantage for the detection of virus might be realized by the use of suitable filters. A search of the literature failed to show examples of the use of membrane filters for the separation and recovery of viruses from sources in nature.

The present study was initiated to explore the possible extension of the principle of membrane filter usage to the recovery of virus from an aqueous source.

MATERIALS AND METHODS

Membrane filters were obtained from two commercial sources.[2] The S & S filters used were of the ultrafine quality, coarse, and medium grade porosity. They were received as either dry, preboiled filters or shipped moist in 20% ethyl alcohol. The Millipore filters were received as dry membranes.[3]

The filter apparatus used was designed for positive pressure filtration and was obtained from the Carl Schleicher and Schuell Company (MD 50–15, 25-ml capacity). The filter holder accepted either 47- or 50-mm filter membranes. It was sterilized (without membranes) by autoclaving at 121 C for 15 min. Positive pressure was supplied from a tank of compressed nitrogen gas. The maximal pressure used at any time was 200 psi.

The virus used in the study was influenza, A/PR8/34 (American Type Culture Collection). Virus retention was studied by passing influenza virus solutions through membrane filters under positive pressure. Following filtration the membranes were reduced to a pulp using sterile mortar and pestle, resuspended, and examined for virus. The extent of virus retention was measured by hemagglutination tests (HA) and chick embryo infectivity titrations (ID_{50}).

Recovery of virus from suspensions containing a mixture of bacteria and virus was examined in the same way indicated above with one exception. Suspensions were first passed through a bacteria-retaining membrane (Millipore HA), the filtrate collected aseptically, and then passed through a virus-retaining membrane.

A few clinical specimens from cases of suspected influenza were obtained for virus isolation. These specimens were treated as indicated for bacteria-virus mixtures. The final membrane suspension was inoculated into monkey kidney monolayers. One milliliter was added to the monolayers and the tubes rotated for 2 or 3 hr at 37 C. The supernatant fluid was then discarded and 1 ml of maintenance medium containing antibiotics added. The cultures were examined periodically for cytopathogenic effects (CPE) and hemadsorption tests (Vogel and Shelokov, 1957) were conducted at 5 and 10 days following inoculation. All tubes showing positive hemadsorption were set aside for passage and hemadsorption-inhibition tests were per-

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[2] The Carl Schleicher and Schuell (S & S) Company, Keene, N. H. The Millipore Filter Corporation, Bedford, Mass.

[3] Virus coarse (VC); virus medium (VM); virus fine (VF).

formed on second-third passage inocula. The method of Shelokov, Vogel, and Chi (1958), employing antisera prepared in rabbits or guinea pigs, was used to identify unknown isolates.

Results

The retention of virus by membrane filters is shown in Table 1. The S & S ultrafine medium and Millipore VM and VF membranes regularly retained virus. Results obtained with the Millipore VC membranes were variable. When the recovery values were compared to the original virus concentration, a difference in apparent recovery of virus was noted, depending on the method of measurement. If the ID_{50} measurement is accepted as the more sensitive, losses of 0.3 to 1.3 logs of virus infectivity occurred where membranes retained virus. The virus recoveries reported in the study were all obtained from pulped membranes. It was observed on several occasions, however, that essentially comparable recoveries could be achieved simply by washing the membrane surface. A given volume of diluent was directed over the surface by means of a pipette, and the washings collected in a Petri dish. The penetration of membrane by virus must have been minimal since virus was recovered from these washings.

The S & S membranes were received in 20% alcohol and could be used directly. The Millipore membranes were received dry and packed between absorbent pads. These membranes were not sterile and it was necessary to sterilize them prior to use. The effect of autoclaving upon their virus-retaining ability was examined to determine the feasibility of sterilizing them in the autoclave. The VM and VF membranes were each placed between several absorbent pads, wrapped in heavy wrapping paper, and autoclaved at 115 C for 15 min. Tests made with autoclaved membranes frequently demonstrated an inability of these membranes to retain virus. It was apparent that sterilization by autoclaving had altered the virus-retaining quality of the membranes. Millipore membranes (obtained through the courtesy of the manufacturer), which had been sterilized by high voltage radiation were used. These membranes uniformly retained virus and proved satisfactory in all respects.

The ability of membrane filters to retain virus was applied to the problem of recovering virus from a source which might also include bacteria. The recovery of virus from bacteria-virus mixtures is shown in Table 2. The results showed that virus could be separated and recovered free from bacteria by preliminary filtration through a bacteria-retaining membrane (Millipore HA) filter. The separation however was accompanied by loss of virus upon the bacterial filter surface. The loss was calculated as that portion of the original virus concentration which had been retained by the Millipore HA filter. Measurements of this retention varied from an average value of 1% as determined by ID_{50} tests, to an average value of 7% for the HA tests. Titrations of the recovered bacteria-free virus obtained from virus-retaining membranes yielded ID_{50} values within 0.7 to 2.0 logs of the original virus titers.

The metal construction of the filter apparatus offered the possibility of a toxic effect exerted by the metal in contact with virus-containing solutions during the course of filtration. This possibility was investigated by allowing a virus suspension to remain in contact with the metal surfaces of the filter apparatus for 2 to 4 hr, then examining the suspension for evidence of virucidal activity. No evidence of a toxic effect could be demonstrated with influenza virus.

The time required for filtration could be decreased appreciably by the use of Celite analytical filter aid. Celite was added to the virus suspension immediately before filtration. Filtration was rapid and virus retention by the membranes remained unaffected with one important exception. The virus recovered was distributed between the Celite layer and membrane. In fact as much and sometimes more virus was found associated with the Celite. The use of Celite greatly

TABLE 1. *Retention of influenza virus by membrane filters*

Filter	Chick red blood cell hemagglutination (titers)			Chick embryo infectivity (ID_{50})		
	Allantoic fluid	Membrane	Per cent recovery (titer/ml × ml suspension)	Allantoic fluid	Membrane	Per cent recovery (ID_{50}/ml × ml suspension)
S & S ultrafine, medium						
1	1,280	3,200	93	$10^{8.8}$	$10^{8.5}$	18
2	2,560	5,120	75	$10^{9.7}$	$10^{8.8}$	5
3	1,280	3,200	93	$10^{8.7}$	$10^{8.5}$	15
4	1,280	3,200	75	$10^{8.7}$	$10^{8.0}$	7
Millipore VC						
1	320	800	94	$10^{7.5}$	$10^{7.0}$	12
2	640	640	37	$10^{7.7}$	$10^{6.0}$	1
3	640	640	37	$10^{8.0}$	$10^{5.5}$	1
4	1,280	1,600	47	$10^{8.3}$	$10^{7.6}$	7
5	640	320	19	$10^{7.7}$	$10^{5.3}$	1
Millipore VM						
1	1,280	3,200	107	$10^{8.0}$	$10^{7.5}$	9
2	640	1,280	75	$10^{9.0}$	$10^{8.5}$	11
3	640	2,560	100	$10^{9.6}$	$10^{8.6}$	3
4	2,560	5,120	86	$10^{8.3}$	$10^{8.0}$	22
5	1,280	3,200	71	$10^{9.8}$	$10^{8.5}$	2
Millipore VF						
1	1,280	3,200	94	$10^{8.6}$	$10^{7.7}$	5
2	1,280	3,200	107	$10^{8.5}$	$10^{7.6}$	5
3	640	2,560	100	$10^{9.5}$	$10^{9.0}$	22
4	1,280	2,560	86	$10^{9.7}$	$10^{9.0}$	9
5	1,280	3,200	94	$10^{9.6}$	$10^{9.0}$	9
6	640	1,280	86	$10^{9.5}$	$10^{9.0}$	14

facilitated the filtration of virus suspensions containing bacteria or tissue debris.

Clinical specimens from students at the University Health Service with symptoms of respiratory virus infections were made available for examination for virus pathogens. Sixteen throat washings were examined by conventional and by membrane filter methods. Monkey kidney monolayers were used in both procedures. In the conventional method, 1 ml of inoculum was added directly to the tissue culture, whereas in the membrane filter procedure the sample was collected on a virus-retaining membrane after first being passed through a bacteria-retaining membrane. The virus-retaining membrane was pulped, centrifuged lightly, and the supernatants inoculated into the tissue culture. The results are given in Table 3.

The membrane filter procedure contributed one more positive isolation than the conventional method. Since the number of analyses was inadequate for purposes of a comparison of the relative efficiency of the two methods, no conclusions are offered. The results did show, however, that monolayers treated with membrane filter-processed inocula were free from the contamination and toxic effects which occurred with conventional method inocula. On the basis of the data shown in Table 3, the results obtained with the membrane filter-treated specimens were at least in agreement with those obtained for the conventional method. These results seemed to justify a more searching examination of the use of membrane filters for diagnostic purposes. The hemadsorbing viruses were identified by hemadsorption-inhibition tests as influenza A_2 strains. Serological examinations of paired serum samples from the patients concerned showed 4- to 6-fold increases in complement fixing antibody titer only with influenza A_2 antigen. The monkey kidney monolayers showing CPE but no hemadsorption yielded transmissible agents whose identity was not fully established. On the basis of positive complement fixation with known adenovirus antiserum, and negative results with known antisera for influenza A, A_1, A_2, psittacosis, Q fever, and parainfluenza, types 1 and 3, an adenovirus was suspected.

Discussion

Membrane filters obtained from two commercial sources were shown to be capable of retaining influenza virus on their surface. Judging from the ease with which virus could be washed off the membrane, the retention consisted of a simple deposition of virus upon the filter surface. In the absence of previous studies on virus retention by membrane filters, the results of the present study provide data on the application of filter usage to virus recovery from aqueous suspensions. For example, separation from bacterial mixtures was accomplished successfully by filtering solutions through bacteria-retaining membranes. Virus could then be collected upon virus-retaining membranes. Although virus was unable to penetrate a membrane with a porosity value less than the virus size, it was retained to some extent by membranes with far greater porosities. This retention indicated that electrostatic forces could not be neglected in virus filtration.

The loss of virus which occurred during filtration may have been the result of mechanical imperfections in the technics used. It was considered more likely, however, that virus inactivation occurred during filtration. This impression was gained from the discrep ancies found between apparent virus recovery a

TABLE 2. *Recovery of influenza virus from mixed virus-bacteria suspensions by means of membrane filters*

Filter	Bacteria-virus suspension			Bacteria-retaining membrane filter				Virus-retaining membrane filter	
				HA titer		Egg ID_{50}			
	Escherichia coli count per ml	HA titer per 0.5 ml	Egg ID_{50} per 0.2 ml	Membrane*	Filtrate	Membrane*	Filtrate	HA titer membrane†	Egg ID_{50} membrane†
S & S ultrafine, medium									
1	84.5×10^7	1,280	$10^{8.0}$	40	640	$10^{6.0}$	$10^{8.0}$	640	$10^{6.6}$
2	73.1×10^7	640	$10^{8.7}$	40	320	$10^{5.5}$	$10^{8.5}$	320	$10^{7.3}$
3	12.8×10^8	1,600	$10^{8.3}$	160	800	$10^{6.7}$	$10^{7.3}$	800	$10^{6.7}$
4	43.2×10^8	2,560	$10^{9.5}$	40	1,280	$10^{7.6}$	$10^{8.7}$	1,600	$10^{7.6}$
Millipore VF									
1	88.1×10^7	1,280	$10^{7.7}$	80	640	$10^{6.3}$	$10^{7.5}$	640	$10^{6.6}$
2	10.1×10^8	2,560	$10^{9.5}$	160	1,280	$10^{7.0}$	$10^{8.8}$	1,600	$10^{8.8}$
3	72.9×10^7	2,560	$10^{9.8}$	320	1,280	$10^{6.7}$	$10^{9.0}$	1,280	$10^{8.0}$
4	68.5×10^6	800	$10^{8.3}$	20	400	$10^{6.0}$	$10^{7.8}$	320	$10^{6.8}$
5	90.9×10^7	400	$10^{8.7}$	80	160	$10^{6.8}$	$10^{7.8}$	160	$10^{6.7}$
6	21.3×10^8	1,280	$10^{9.0}$	40	640	$10^{7.6}$	$10^{8.5}$	800	$10^{7.0}$
7	77.6×10^7	640	$10^{8.5}$	40	320	$10^{6.7}$	$10^{8.0}$	320	$10^{6.6}$

* Membrane resuspended to initial volume of bacteria-virus suspension.

† Membrane resuspended to volume of filtrate obtained from bacterial membranes filter filtration.

measured by hemagglutination and chick embryo infectivity tests. Since 10^6 to 10^7 ID_{50} values are considered necessary (Henle and Henle, 1949; Fazekas de St. Groth and Cairns, 1952) to obtain visible hemagglutination, the consistently greater hemagglutination end points indicated that a portion of the virus population had been rendered noninfective. According to the results obtained in the present study, as many as 100 ID_{50} doses were lost.

TABLE 3. *Virus isolation obtained in monkey kidney monolayers by conventional and membrane filter methods*

Specimen	Conventional method				Membrane filter			
	Hemadsorption	CPE	Contamination	Toxicity	Hemadsorption	CPE	Contamination	Toxicity
1	0	0	0	0	0	0	0	0
2	+	+	0	0	+	+	0	0
3	0	0	+	+	0	0	0	0
4	+	+	0	0	+	+	0	0
5	0	0	0	+	+	+	0	0
6	0	0	+	+	0	0	0	0
7	0	0	+	+	0	0	0	0
8	0	0	0	+	0	+	0	0
9	0	0	0	0	0	0	0	0
10	+	+	0	0	+	+	0	0
11	0	0	0	+	0	0	0	0
12	0	0	0	0	0	+	0	0
13	0	0	0	+	0	0	0	0
14	+	+	0	+	+	+	0	0
15	0	0	0	0	0	0	0	0
16	0	0	0	+	0	0	0	0

The successful isolation of Asian influenza strains indicated a potential application of the technic to diagnostic purposes. The freedom from bacterial contaminants and toxic effects, shown by membrane filter-treated inocula, offer distinct advantages where tissue culture methods are involved.

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