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Title	Concentration of Measles Virus and Its Complement Fixing Antigen
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Concentration of Measles Virus and Its Complement Fixing Antigen

High virus yields of about 10^6 $TCID_{50}$ per 0.2 ml have been constantly obtained in FL cell cultures infected with the Toyoshima strain of measles virus which was isolated and established in FL cells.[1, 2] When the virus was stored at -20°C without any fractionation it was rather stable and almost no decrease of infective titer was detected during three and a half months. Therefore studies were made on fractionation of the virus. Since there is little data on centrifugation of this virus we studied its concentration by centrifugation.

Original virus pools were prepared as follows; infected FL cell cultures were harvested during the period of maximal cytopathic change. The pH was adjusted

Table 1. Centrifugation of Measles Virus

No.	Centrifugation		Sample		Infectivity log $TCID_{50}$ /0.2ml	CF-antigen dilution
	average g	time min.	specimen	volume/original vol.		
1.			original	—	5.1	8
	53,800[a]	90	sup.	—	<3.8	<1
			ppt.	1/13	6.3	64
2.			original	—	6.0	16
	53,800[a]	90	sup.	—	3.5	4
			ppt.	1/13	6.5	128
	41,200[b]	90	sup.	—	2.8	4
			ppt.	1/12	nt	128
	20,200[b]	90	sup.	—	<2.8	8
			ppt.	1/12	6.8	64
	10,300[b]	90	sup.	—	<2.8	4
			ppt.	1/12	6.8	128
	6,590[b]	90	sup.	—	4.2	4
			ppt.	1/12	6.6	128
3.			original	—	6.3	16
	6,590[b]	90	sup.	—	3.7	1
			ppt.	1/12	6.8	128
	3,230[b]	90	sup.	—	4.6	2
			ppt.	1/12	6.8	128
	1,650[b]	90	sup.	—	4.8	4
			ppt.	1/12	6.6	64

original : supernatant of original virus pool after centrifugation at 3,000—5,000 rpm for 15—20 min.
Centrifuged in a RP 30 rotor (a) and in a RP 40 (b) of a Hitachi preparative ultracentrifuge Model 40P.
nt : not tested.
sup. : top 1/5 of supernatant fluid.
ppt. : pellet resuspended in maintenance medium.

to 7.0–8.0 with 7 per cent sodium bicarbonate and the samples were frozen at -20°C. After a suitable time, the samples were thawed, pooled and stored at -20°C as before. The virus was titrated by inoculating 3 FL tubes with 0.2 ml of each dilution at half log intervals. Cultures were examined after 14 days. CF antigen was titrated by a modification of Colmer's method. 0.2 ml of serial two fold dilutions of antigen were mixed with 0.1 ml of phosphate buffered saline containing 2 units of complement and 0.1 ml of standard antibody. After overnight fixation at 4°C, the mixture was mixed with 0.1 ml of sensitized ox red cells and the highest antigen dilution which failed to show complete hemolysis after one hour's incubation at 37°C was taken as the antigen titer.

Pooled samples were thawed immediately before fractionation and centrifuged (3,000 rpm, 15–20 min.). The supernatant fluid was used as starting material. After each centrifugation the top one fifth of the medium and pellet, resuspended in maintenance medium, were tested for their infectivity and antigenicity.

As shown in Table 1. even by centrifugation at an average of 1,650 *g* for 90 min. a considerable quantity of the virus and CF antigen had been sedimented. By centrifugation at 1,400 *g* for 20 min. there was no remarkable decrease in virus or CF antigen in the fluid though 50 per cent of the CF antigen had been sedimented with the cell debris after centrifugation at 2,000 rpm for 10 min.

The particle size of this virus as suggested by ultrafiltration[3] and the core size sensitive to ionizing radiation[3] are compatible with this results. On the other hand CF antigen was sedimented at a much lower centrifugation rate than that expected from the radiation study[3] or from the previously reported data on centrifugation of CF antigen.[4] It is still uncertain whether this caused by the association of CF antigen with some larger particles or by its self-aggregation.

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