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# STANDARDIZED METHOD OF SENDAI VIRUS PRODUCTION FOR BIOLOGICAL ASSAYS

P.-P. PASTORET, *G. BURTONBOY[1]), *M. E. LAMY, *M. Cl. VAN DIJCK, F. SCHOENAERS

Laboratory of Virology, Faculty of Veterinary Medicine, University of Liège, 1070 Bruxelles; and *Laboratory of Virology, Faculty of Medicine, Catholic University of Louvain, 1200 Bruxelles, Belgium

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*Summary.* — A method for the production and purification of Sendai virus is described.

*Key words: Sendai virus; purification; assay*

We are describing a technique making it possible to purify Sendai virus in a single step from large quantities of crude allantoic fluid, with a good yield and in satisfactory conditions of bacterial sterility and safety.

The strain of Sendai virus and the L-cell line used were kindly supplied by Dr. Billiau, REGA Institute, Catholic University of Louvain. The cells were grown in Minimal Essential Medium (MEM), Earle's buffered saline solution, with reduced bicarbonate, non-essential amino acids, penicillin and streptomycin and supplemented with 10% calf serum. The Sendai virus was produced in SPF eggs. The saline solution used for the haemagglutination (HA) reaction contained 9 g NaCl, 100 mg $MgCl_2 . 6 H_2O$ and 41 mg $CaCl_2 . 2 H_2O$, to one liter of distilled water.

*Production of Sendai virus.* Before beginning to quantify the parameters of the viral production, the virus was serially passed in eggs, with regularly increasing dilutions of the inoculum. This procedure was continued until the HA titres obtained were consistently satisfactory for all the harvests (8192). The eggs were inoculated by the allantoic route with 0.2 ml of infected allantoic fluid diluted to $10^{-7}$ in Hanks' solution containing 5% lactalbumin hydrolysate (LAH). After incubation at 35° C for 78 hours, the allantoic fluids were harvested, pooled and clarified by centrifugation at 4000 rev/min for 10 minutes at 4°C; this constituted the viral stock which was tested for HA and infectivity.

*Hen erythrocytes for the HA reaction.* Hen's blood was collected by cardiac puncture into Alsever's fluid and washed three times in saline. Then 0.4 ml of the last pellet was suspended in saline and after estimating the cell count by a Coulter counter, the suspension was adjusted to contain $20 \times 10^6$ red blood cells per ml. This concentration was chosen because it enabled to obtain the highest HA titre of Sendai virus and at the same time gave a clear reading of the reaction.

*Haemagglutination reaction.* The viral suspensions were diluted with the COOKE microtiter (0.025 ml), and 0.025 ml of the hen's red blood cell suspension was added to each virus dilution The results were read after 30 minutes of contact at room temperature.

*Titration of infectivity.* a) By *haemadsorption:* We used a modification of the technique described by Kashiwazaki *et al.* (1965). The L cells were grown on Linbro macroslides 16 mm in diameter, using MEM with 10% calf serum. Each slide was seeded with $5 \times 10^5$ cells and cultured in incubator with 5% $CO_2$ until formation of a monolayer. Then the cells were rinsed twice with PBS and inoculated with 0.2 ml per slide of each virus dilution. Tenfold dilutions of virus were made in Hanks' solution containing 5% LAH. The cells with virus were incubated at 37° C for one hour, then twice rinsed with PBS, covered with Hanks' solution containing 5% LAH and incubated further at 37° C. After 24 hours, they were twice rinsed in chilled (4° C) PBS and

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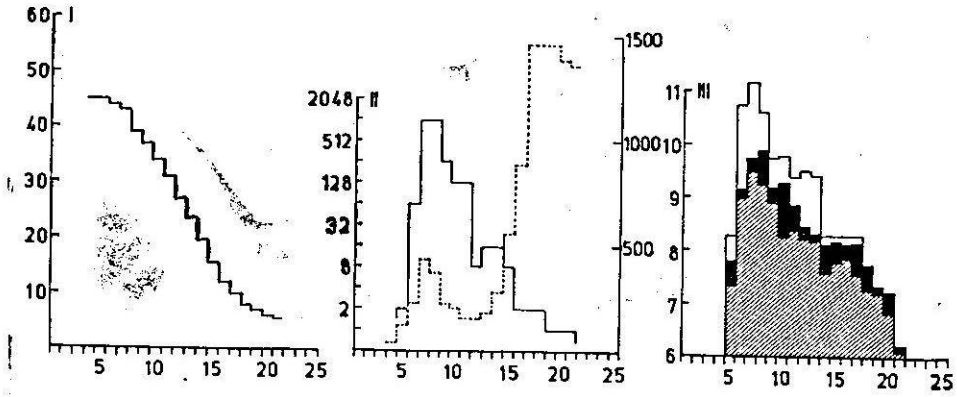


**Fig. 1.**

Partial purification of Sendai virus on a sucrose gradient

Abscissae: fraction number (each fraction = 1 ml)

I — Sucrose per cent

II — HA titre (left ordinate; ————) of fractions diluted 1 : 10 and protein contents in μg/ml (right ordinate; — — — —)

III — Number of physical particles per ml (empty columns) or PFU/ml as determined by IF (black columns) or haemadsorption (shaded columns) techniques; $\log_{10}$ values

covered with 1.5 ml of a 1% suspension of guinea pig red blood cells (at 4° C in Hanks' solution with 5% LAH). After two hours at 4° C, they were rinsed four times in cold PBS. The results were read while the cells were covered by PBS with a Nikon reverse microscope at a magnification of × 100, focusing on the red cells. For each slide, cells covered with red cells were counted in 10 random microscope fields. The calculated mean was multiplied by a factor (previously established for our conditions of reading) which enabled the result obtained to be extrapolated to that of the whole slide. The infectivity titre was calculated from the results obtained with the slide showing 10 to 12 haemadsorption areas per microscope field. The final result was expressed in the number of plaque forming units (PFU) per ml. b) By *immunofluorescence* (IF). The L cells were grown and inoculated in the same way as for haemadsorption. Twenty-four hours after inoculation, the cells were twice rinsed in PBS and fixed with ethanol for 10 minutes. Thereafter they were rinsed in PBS and allowed to react for 30 minutes at 37° C with 0.4 ml of anti-Sendai rabbit serum at a 1 : 32 dilution. After rinsing twice in PBS, the cells were put in contact, for 30 minutes at 37° C, with 0.4 ml of goat serum anti-rabbit IgG, conjugated with fluorescein isothiocyanate (HYLAND), diluted at 1 : 60. The cells were then rinsed four times in PBS. The number per microscope field of cells presenting cytoplasmic fluorescence was determined by a Leitz fluorescence reverse microscope. The infectious titre was calculated as for haemadsorption.

*Density gradient centrifugation.* To purify the virus in a one-step procedure, we have modified the method described by Smith (1967). For reasons of safety (Giles and Ruddle, 1973), the virus was centrifuged in polycarbonate tubes of 30 ml capacity fitted with screw caps, sterilized by autoclaving. A 15 to 45% sucrose gradient in distilled water was formed by a mixer of the type used by Smith (1967). Three ml of a 60% solution of sucrose in distilled water was overlaid with 12 ml of the gradient and on this was layered about 15 ml of the virus suspension. The stoppered tubes were centrifuged in a fixed-angle rotor of a Martin-Christ Omega II ultracentrifuge at 36,000 rev/min for 3 hours at 4° C. Fractions were collected by an LKB collector; their density was measured with a refractometer.

*Electron microscopy.* Dialysed fractions were examined by negative staining according to the pseudoreplica method recommended by Smith (1967). Particles were counted on photographs at a magnification of about 15,000.

*Protein content* in the samples was estimated by Lowry's method, if necessary, after dialysis.

*Comparison between the number of PFU obtained by haemadsorption and IF technique.* In our conditions of work (24 hours of incubation before reading), the titre obtained by the IF technique was regularly higher (Fig. 1). Our results confirmed those of Kashiwazaki *et al.* (1965). The IF technique

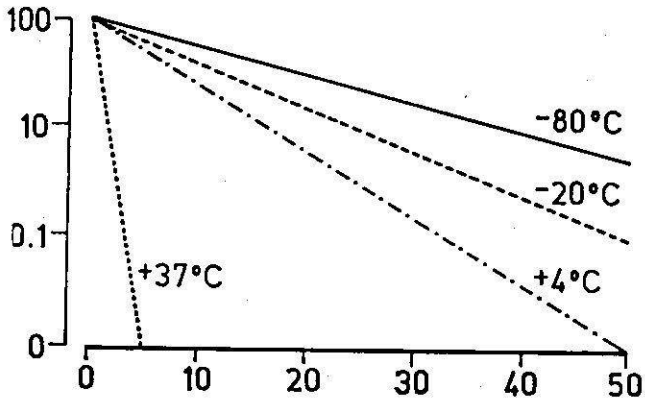


**Fig. 2.**

Decay of Sendai virus infectivity at various temperatures
Abscissa: days; ordinate: infectivity %
Regresion lines computed from data at 0, 1, 2, 3, 4, 8, 15, 21 and 60 days.

furthermore presented the advantage of its relative simplicity, specificity, and of an easy reading.

*Density gradient centrifugation.* Fig. 1 illustrates the results obtained. We recovered 25% of the physical particles from the total amount before centrifugation. The protein contents was reduced to only 2% of the initial amount. The viral particles were collected from the opalescent band situated at a level where the density ranged near 1.18.

*Relationship between the number of physical particles in a viral suspension and its HA titre.* In our conditions of work, the virus: red cell ratio was 1.6 for haemagglutination to take place. With good standardized working methods, one may thus estimate the number of physical particles in a viral suspension from its HA titre.

*Decay.* The samples were kept at — 80° C, since previous experiments have shown a loss of infectivity at various temperatures (Fig. 2).

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