Production of Hemagglutinating Antigens of La Crosse Virus by Polyethylene Glycol Precipitation

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Hemagglutination titers of La Crosse virus antigens prepared in BHK-21 suspension cell cultures were substantially increased by precipitating the antigens with polyethylene glycol.

La Crosse (LAC) virus is now recognized as a major cause of arboviral encephalitis in the United States (6). Diagnosis of LAC infection is typically accomplished by demonstration of diagnostic increases in antibody titer between acute- and convalescent-phase serum specimens (7). Commonly used tests include virus neutralization, hemagglutination (HA) inhibition, and complement fixation. Problems are associated with each of the tests. Complement fixation is considerably less sensitive than the other tests and detects only short-lived antibody (5). Virus neutralization requires the maintenance of tissue culture capability and sophisticated virus containment facilities. Production of HA antigens has also been difficult. Mouse brain preparations frequently exhibit HA activity only after sonic treatment and use of high-salt molarity adjusting diluents (2). The use of suspension cell culture technique (3) was a major improvement in the production of LAC HA antigens, but, in our hands, has not consistently resulted in high HA titers. We report here a modification of the suspension cell culture technique for the reliable production of high-titered LAC HA antigens.

LAC virus (which had been passed seven times in baby mice and then six times in BHK-21 cell cultures) was inoculated into BHK-21 cells to prepare virus stocks. Culture fluids were harvested at 3+ to 4+ cytopathic effect, centrifuged, and stored at -70°C until used. The stock virus titer was 4.1×10^7 plaque-forming units per ml. BHK-21/13s cells were kindly provided by Adrian Chappell, Centers for Disease Control, Atlanta, Ga. Monolayers were grown at 37°C in 150-cm² flasks of medium containing 80% Hanks balanced salts, 10% tryptose phosphate broth, and 10% newborn calf serum. When cell sheets became confluent, five flasks were trypsinized, and cells were suspended in 1 liter of medium and placed in a Spinner culture bottle. These suspension cultures were grown at 37°C with constant stirring for 24 h before infection with the virus. The method of Chappell et

al. (3) with serum-free medium was used to infect the cultures. At 6-h intervals, 2-ml samples were removed from the cultures, centrifuged at $755 \times g$ for 10 min, and tested for HA activity. Plaque assays were performed on a portion of the sample to monitor infectious virus titers.

The hemagglutinating antigens were harvested after centrifugation at $755 \times g$ for 20 min. The supernatant fluid was removed and stored. In some instances the cell pellet was suspended in 1 liter of fresh serum-free medium and reincubated as before. Antigens were subsequently harvested from these resuspended cultures.

HA and HA inhibition assay procedures were those of Clarke and Casals (4) adapted to microtiter. Sera for HA inhibition tests were acetone treated and adsorbed with goose erythrocytes. Antisera to LAC virus, to three additional California group viruses (or subtypes) (i.e., snowshoe hare, trivittatus, and Jamestown Canyon), and to yellow fever virus were used in the HA inhibition tests.

A portion of each antigen preparation was concentrated by polyethylene glycol (PEG) precipitation. NaCl was added (2.2%, wt/vol) to the cell culture fluid, and the preparation was stirred until the NaCl had dissolved. PEG 6000 was then added to a concentration of 10%. After the PEG 6000 dissolved, the suspension was refrigerated at 4°C overnight in the centrifuge tubes. The preparation was then centrifuged at $12,000 \times g$ for 30 min at 4°C . The pellet was suspended in a minimum volume of cold sterile STE buffer [NaCl, 5.84 g/liter; tris(hydroxymethyl)amino-methane-hydrochloride, 1.21 g/liter; ethylenediaminetetraacetic acid, 0.37 g/liter, pH 7.2]. The new suspension was then centrifuged as before, and the supernatant fluid was stored at -70°C .

HA activity was detected in the fluids of the BHK-21 suspension cultures in all four trials. HA titers were 1:8 in one trial and 1:16 in the others (Table 1). Peak antigen production occurred at approximately 48 h postinfection. PEG

TABLE 1. *Titers of LAC virus spinner culture antigens before and after PEG precipitation*

Antigen prepn	HA titer (reciprocal dilution)		HA titer increase (fold)
	Before PEG ppt ^a	After PEG ppt	
1	8	32	4
2	16	512	32
3	16	256	16
4	16	64	4
5 (resuspended)	4	16	4

^a ppt, Precipitation.

precipitation greatly increased the HA titer of each preparation (Table 1). Titer increases of 32-fold could be obtained depending on the volume of the buffer used to suspend the pellet. There was no apparent increase or decrease in the specificity associated with PEG precipitation. HA inhibition titers for the serologically related California group viruses were similar regardless of the preparation of the antigen.

Enhancement of HA titer by sonic treatment was attempted. Both pre- and post-PEG precipitation preparations in each trial were sonicated by the technique of Ardoin and Clarke (1). Sonication of the antigens did not enhance HA activity. In fact, in all but one preparation, sonication reduced the HA titer.

Resuspension of infected cells after harvesting of the initial antigen resulted in production of a second antigen. Titers did not exceed 1:4, but with PEG precipitation the titer was increased (Table 1). Resuspension of cells in an attempt to produce a third antigen preparation was unsuccessful.

The use of BHK-21 suspension cell cultures and PEG precipitation and concentration of the resultant antigen preparations provides a consistent method for the production of LAC HA antigens. There is no apparent loss in specificity of the antigen because of concentration. Resuspension of infected cells and subsequent production of a second HA antigen preparation should lessen the cost of the method.

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