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Production and Use of a Hemagglutinin for Detecting Antibody to Jamestown Canyon Virus

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A procedure was developed for producing a hemagglutinin for the California serogroup (family *Bunyaviridae*, genus *Bunyavirus*) virus Jamestown Canyon, a human pathogen. Serum samples from humans putatively infected with this virus or with La Crosse virus were tested by hemagglutination inhibition. Each antigen detected antibody to the respective virus, with little cross-reactivity. These results suggest that both antigens should be used when the hemagglutination inhibition test is applied to the diagnosis of human infections with California serogroup viruses in North America.

California serogroup viruses (family *Bunyaviridae*, genus *Bunyavirus*) cause human disease in North America (6, 9, 10, 13). Initially, La Crosse (LAC) virus was considered the principal etiologic agent, but subsequent findings implicated snowshoe hare and Jamestown Canyon (JC) viruses as human pathogens. As for most arbovirus infections, the hemagglutination inhibition (HI) test is least serotype specific and the neutralization (N) test is most serotype specific for diagnosis of infections caused by these viruses. Because California serogroup viruses are so closely related antigenically (4), serodiagnosis of infections caused by these viruses relies on a combination of HI, complement fixation, and serum dilution-plaque reduction N tests (4) and, for LAC virus infections, immunoglobulin M antibody-capture enzyme-linked immunosorbent assay (5) as well. HI is only partially satisfactory as a screening test for antibody to LAC virus (3). LAC hemagglutinin (7) does not detect antibody to JC virus (10), probably because these viruses are not members of the same antigenic complex (6). The HI test is a relatively simple test that can be used in most laboratories performing serodiagnosis. Epidemiologic evidence (10; P. R. Grimstad, *in* T. P. Monath, ed., *Epidemiology of Arthropod-Borne Viral Diseases*, in press) suggests that human infection with JC virus frequently occurs in the upper midwestern United States, and severe clinical illness can result (11). Therefore, JC virus hemagglutinin would be useful.

Until now, the only method for producing a JC virus hemagglutinin has been that of Ardoin (cited in reference 2). The details of that method were not given but were stated as being those used for production of other bunyaviruses. One of us (N.K.) developed and described a procedure for preparing hemagglutinins for California serogroup viruses from infected BHK-21 cell cultures. Although this procedure was applied to the production of a JC virus hemagglutinin, results were not consistently successful and JC virus hemagglutinin was not used in the study subsequently reported (12). One of us (H.A.) has successfully prepared such a hemagglutinin from many non-reference strains of JC virus by modifying this method. Apparently, the prototype strain (61V-2235) is not one from which a hemagglutinin can be made without applying other modifications. Strain Mn 256-260 had been isolated from *Aedes* species mosquitoes collected in 1979 in Manitoba Province, Canada, and was subsequently shown to be serologically identical to prototype JC virus in enzyme-linked immunosorbent assays (1) and N tests. We used this isolate to prepare hemagglutinins for the tests reported here.

Vero cells were grown to monolayer cultures in 150-cm^2 flasks and infected with a multiplicity of infection of 0.1 to 0.5. Infected cultures were maintained at 37°C with Eagle minimum essential medium containing 0.4% bovine albumin and antibiotics. When 75 to 100% of the cells showed cytopathic effects, the supernatant fluids were pooled and clarified by low-speed centrifugation. The virus-containing supernatant was adjusted to 0.5 M NaCl (2.04 g of NaCl per 100 ml of supernatant), and 6% (wt/vol) polyethylene glycol 8000 was added. The mixture was stirred at 4°C for 3 to 4 h or overnight. The treated fluid was then centrifuged at 15,000 × *g* for 30 min at 4°C, and the supernatant was discarded. Pelleted virus was suspended in 1/20 of the original volume with Eagle minimum essential medium containing 3% heat-inactivated, kaolin-adsorbed (8) fetal bovine serum and allowed to stand at room temperature for several hours.

The hemagglutinin was inactivated by Tween 80-ether treatment. Tween 80 diluted in phosphate-buffered saline (pH 7.2) was added to a final concentration of 1%, and the mixture was stirred at room temperature for 15 min. One-half volume of cold, anesthesia-grade ether was added, and the mixture was stirred at room temperature for 15 min and then centrifuged at 4,000 × *g* for 15 min at 4°C. The treated virus suspension was removed from below the ether phase, and residual ether was allowed to evaporate by stirring it in an open container for 15 to 30 min. An equal volume of reagent-grade Freon was added, and the mixture, shaken by hand every 10 min, was held at 4°C for 30 min. Finally, this mixture was centrifuged at 2,000 × *g* for 10 min at 4°C, and the Freon-treated virus suspension (supernatant) was removed, dispensed in aliquots, and stored at −70°C.

The antigen was tested by the standard method of Clarke and Casals (8). The pH range for agglutination of goose erythrocytes was 5.65 to 6.2, with an optimum pH of 5.7; the endpoint titer of the antigen was 80.

This hemagglutinin and one prepared with prototype LAC (original strain) virus (7) were used to test 44 serum samples

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TABLE 1. Results of HI and serum dilution-plaque reduction N tests[a]

Serum no.	Titers in:			
	HI test		N test	
	JC	LAC	JC	LAC
1	10	–[b]	64	–
2	40	–	32	–
3	160	10	64	2
4	20	–	64	–
5	10	–	16	–
6	40	–	128	–
7	20	–	32	–
8	40	–	32	–
9	40	–	128	4
10	20	–	16	–
11	10	–	16	–
12	20	–	64	–
13	–	–	8	–
14	10	–	16	–
15	20	–	64	–
16	–	–	8	–
17	–	–	8	–
18	10	–	32	–
19	>640	160	>1000	32
20	80	10	256	16
21	80	–	256	4
22	40	–	128	4
23	40	–	64	–
24	20	–	32	–
25	10	–	64	–
26	–	80	80	640
27	–	20	20	320
28	160	640	160	640
29	–	40	40	640
30	20	320	80	640
31	–	–	–	40
32	–	40	160	640
33	–	20	80	640
34	20	320	160	640
35	160	5,120	640	320
36	–	10	20	640
37	–	40	320	640
38	20	80	320	640
39	–	10	160	640
40	–	80	640	640
41	–	20	–	40
42	–	20	10	320
43	–	20	20	160
44	10	160	40	640
45	40	1,280	80	640
46	–	10	–	20
47	–	–	–	20
48	–	10	–	80
49	–	40	–	160
50	20	160	20	160
51	–	–	–	–
52	–	–	–	–
53	–	–	–	–
54	–	–	–	–
55	–	–	–	–

[a] Serum samples 1 to 25 were from humans with evidence of infection with JC virus, serum samples 26 to 50 were from humans with confirmed infection with LAC virus, and serum samples 51 to 55 were from humans with no N antibody to either virus.

[b] –, Titer of <10 by HI test or <2 or <10 by N test.

from adult humans with N antibody attributed to JC virus infection (10), 25 serum samples from adult humans with N antibody attributed to LAC virus infection, and 10 serum samples from adult humans with no N antibody to either of these viruses (Table 1).

All serum samples with HI antibody to JC virus also had N antibody to JC virus; all serum samples with HI antibody to LAC virus had N antibody to LAC virus. Of 25 serum samples with N antibody to JC virus, 3 did not have HI antibody to JC virus; of 25 serum samples with N antibody to LAC virus, 2 did not have HI antibody to LAC virus. Of 25 serum samples from persons with putative JC virus infections, 19 had HI antibody to JC virus but not to LAC virus; of 25 samples from persons with confirmed LAC virus infections, 15 had HI antibody to LAC virus but not to JC virus. Alternatively, serum samples from individuals with confirmed LAC virus infections were more cross-reactive with JC virus in N tests. Clearly, the JC virus hemagglutinin used was as specific in detecting antibody to JC virus as the LAC virus hemagglutinin was in detecting antibody to LAC virus.

The results provide further evidence that the use of LAC virus hemagglutinin is not sufficient to detect antibody to all California serogroup viruses occurring in North America and that JC virus hemagglutinin is required to detect HI antibody to JC virus. Use of only JC virus in a screening serum dilution N test (10) detected all human sera with HI antibodies to LAC virus. In addition, human sera with N antibody to trivittatus virus (also a California serogroup virus), none of which had reacted in standard HI tests with LAC virus hemagglutinin (P. R. Grimstad, unpublished results), have been readily detected (with only JC virus in the screening N test). Although the immunoglobulin M antibody-capture enzyme-linked immunosorbent assay and the N test are the serologic tests of choice for diagnosis of California serogroup virus infections, the use of both JC and LAC virus hemagglutinins appears to be an alternative technique for serosurveys and, under certain circumstances, for primary serodiagnosis of infections caused by these viruses.

JC virus has been associated with variable clinical symptoms in humans (10, 11; Grimstad, in press) and is found essentially throughout North America (6), in contrast to LAC virus, which has a rather limited geographic range (Grimstad, in press). Therefore, clinicians and other public health workers in North America outside the recognized geographic range of LAC virus should consider JC virus infections in differential diagnoses of individuals with febrile illness having onset from mid-April through late November.

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