Rapid Preparation of Hemagglutinins of Togaviruses from Infected Cell Culture Fluids

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Antigens in infected cell culture fluids can be easily concentrated by polyethylene glycol precipitation to yield suitable hemagglutinating and complement-fixing antigens for several togaviruses.

Although accumulation of hemagglutinin (HA) of Japanese B encephalitis (JBE) virus in infected cell culture fluids has been described (5, 8), little or no HA activity has been recovered for other togaviruses (9, 10). [The term togavirus was recommended by the Subcommittee of Vertebrate Viruses (1) to the International Committee on Nomenclature of Viruses for the arboviruses belonging to serogroups A and B.] We routinely prepare HA of slow-growing group B and other togaviruses for HA inhibition (HI) tests by concentrating infected culture fluids by using polyethylene glycol (PEG) precipitation (7, 15).

Confluent monolayers of PS cells or Vero cells are inoculated with 1/100 infected mouse brain stock for 1 hr, washed, and incubated at 37°C in Eagle’s medium or medium 199 supplemented with 0.2% bovine serum albumin. Culture methods, cell lines, and virus strains employed were described previously (6, 13, 14). When cytopathic effect develops, harvested fluids are clarified at 10,000 x g for 10 min at 4°C and then made 8% with respect to PEG 6000 (Koch-Light Ltd., Colnbrook, Bucks, England) by adding 40% (w/v) stock solution at pH 7.8 in Hanks salt solution. After 1 hr at 4°C, virus is sedimented at 10,000 x g for 30 min; the fluid is decanted and drained, and pellets are resuspended to 1/50 volume in borate-buffered saline (pH 9.0) containing 0.2% bovine serum albumin. Sodium azide to 0.1% may be added as a preservative, and the preparation may be rendered noninfectious by incubation with an equal volume of 0.2 M tris(hydroxymethyl)aminomethane-0.2 M β-propiolactone (BPL; Koch-Light) at 4°C for 4 days.

Omission of serum from the culture medium eliminates the problem of nonspecific inhibitors (from serum) in the HA products. Furthermore, the precipitate is less bulky and more easily dispersed. Concentrated HA (with no BPL) comprises mainly infectious virus plus a small component of SHA or slow-sedimenting HA (11, 12); note also the CF activity in Fig. 1. Amino acid labeling and electrophoresis (15) showed that all major proteins are virus-specific. An average recovery of 60% of infectivity was obtained in 18 experiments with JBE, Kunjin, Murray Valley encephalitis, St. Louis encephalitis, and West Nile viruses. Routinely 100% of the HA activity and 40% of infectious virus (and associated radioactivity) were recovered after a second precipitation (Table 1).

Of 16 togaviruses tested, 14 yielded useful HA antigens, with the pH optimum usually lower than that of the corresponding mouse brain antigen (Table 2). In contrast to mouse brain HA of low titer, cell-derived preparations always gave clear hemagglutination patterns; infected culture fluids with no detectable HA could be treated to produce useful HA preparations, with titers as high as 1:32 (Table 2).

These HA preparations are readily prepared from culture fluids in about 2 hr for use in Microtiter HI tests in which only small volumes are required. Residual PEG has no apparent effect on HA patterns or on HI antibody titers, and the observed antibody specificities are identical, or slightly greater than, those obtained with HA antigens prepared more laboriously by sucrose-acetone extraction of suckling mouse brain pools. The HA and CF activities remain stable for at least 3 months at 4°C. The method described offers an easier method than the standard procedures (4) and also is a practical alternative to maintenance of a mouse colony for production of togavirus antigens.

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Fig. 1. Sedimentation analysis of $^{32}$P-labeled Kunjin virus after concentration by PEG precipitation from culture fluids of infected Vero cells. The resuspended concentrate was sedimented through a 5 to 25% sucrose density gradient in an SW 25.1 rotor at 25,000 rev/min for 2 hr. Direction of sedimentation is from right to left. (A) Distribution of radioactivity (●) and of HA (■) (HA titer per 0.025 ml assayed by Microtiter method); SHA denotes peak of slow sedimenting HA. (B) Distribution of CF antigen detected by a Microtiter CF test (●) with excess specific antibody (CF titer per 0.025 ml).

Table 1. Recovery of Kunjin virus during concentration by two cycles of precipitation by 8% polyethylene glycol

| Material                  | Vol (ml) | Total counts/min | Total PFU$^a$ | HA (per 0.025 ml) |
|---------------------------|----------|------------------|---------------|-------------------|
| Original fluid$^b$        | 300      | $180 \times 10^6$| $3.3 \times 10^{10}$ | —                 |
| Virus resuspended         |          |                  |               |                   |
| After first precipitation | 8        | $6 \times 10^4$   | —             | —                 |
| After second precipitation| 1.6      | $3.6 \times 10^4$ | $1.4 \times 10^{10}$ | —                 |
| Original fluid$^c$        | 1,200    | —                | —             | 1/4               |
| Virus resuspended         |          |                  |               |                   |
| After first precipitation | 30       | —                | —             | 1/128             |
| After second precipitation| 1.2      | —                | —             | 1/8,192           |

$^a$PFU, plaque-forming units.

$^b$Pooled culture fluids were harvested from PS cells infected with Kunjin virus and labeled with 1 $\mu$Ci of $^3$H-leucine per ml and 1 $\mu$Ci of $^3$H-tyrosine per ml during growth.

$^c$Not tested.

$^d$Similar to (b) but unlabeled.
### Table 2. Togavirus cell culture hemagglutinating antigens

| Virus                 | Strain | Group | Harvested (days post-infection) | HA titer | pH optimum* | Titer* |
|-----------------------|--------|-------|---------------------------------|----------|-------------|--------|
|                       |        |       | TC HA                           | SMB HA   | TC HA       | SMB HA |
| Group B               |        |       |                                 |          |             |        |
| Murray Valley encepha-| MRM 60 | B     | 2                               | 1/4       | 6.6         | 1/320  |
| listis                |        |       |                                 |          | 6.6         | 1/1,024|
| Kunjin                | MRM 61C| B     | 2                               | 1/8       | 6.4         | 1/640  |
| St. Louisencepha-     | Hubbard| B     | 2                               | 1/2       | 6.4         | 1/80   |
| listis                |        |       |                                 |          | 6.8         | 1/64   |
| Japanese Bencepha-    | Nakayama| B   | 2                               | 1/8       | 6.4         | 1/640  |
| listis                |        |       |                                 |          | 6.6         | 1/5,260|
| West Nile             | Sarafend| B   | 2                               | 1/8       | 6.2         | 1/320  |
| Alfuy                 | MRM 3929| B    | 5                               | 1/2       | 6.6         | 1/128  |
| Dengue type 1         | Hawai| B      | 7                               | <1/2     | 6.4         | <1/2   |
| Dengue type 2         | New Guinea C| B | 3                               | 1/6     | 6.2         | 1/1280 |
| Dengue type 3         | H-87  | B      | 6                               | <1/2     | 6.2         | 1/16   |
| Dengue type 4         | H-241 | B      | 5                               | <1/2     | 6.2         | 1/32   |
| Yellow fever          | 17D    | B      | 3                               | <1/2     | 6.4         | 1/32   |
| Edge Hill             | C 281 | B      | 9                               | <1/2     | 7.0         | 1/4    |
| Stratford             | C 338 | B      | 10                              | <1/2     | 7.2         | <1/2   |
| Kokobera              | MRM 32 | B     | 4                               | 1/2      | 6.6         | 1/128  |
| Group A               |        |       |                                 |          |             |        |
| Sindbis               | MRM 39 | B     | 2                               | 1/8      | 6.2         | 1/512  |
| Ross River            | T 48  | B      | 2                               | 1/4      | 5.75        | 1/128  |

*a Group B viruses were grown in PS cells and group A were grown in Vero cells.

*b Comparison of cell culture (TC) with infected mouse brain (SMB) pools.

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