Stability of a Modified, Live Panleucopenia Virus Stored in Liquid Phase

G. M. POOLE

The Lilly Research Laboratories, Greenfield, Indiana 46140

Received for publication 24 July 1972

It has been shown that a modified, live panleucopenia virus is quite stable when stored in the medium in which it was produced.

The literature has referred to the stability of the virus causing panleucopenia in the cat family (1, 2). The work reported here extends the knowledge of that stability. During experiments with this virus (in developing fluorescent-antibody testing procedures and later while investigating growth characteristics), studies were done on the in vitro stability of this virus. Fluid samples were tested for viability after storage at room temperature in glass and plastic containers for periods of up to 5 months. The number of viable virus particles (median tissue culture infective dose) remained unchanged, leading to further investigations of virus stability, the subject of this report.

A tissue culture of feline kidney origin was used exclusively for growth of the virus and titration. Those cells used for growth of the virus were at a passage level of 100. For virus titration, cells were at a passage level of 45 to 50.

The medium used was Eagle minimum essential medium modified with sodium bicarbonate, nonessential amino acids, fetal calf serum, glutamine, and antibiotics. This medium was essentially the same for replication of the virus and for testing viability.

The 75th virus passage of a strain of feline panleucopenia virus was produced in 64-ounce Owens-Illinois bottles with 1 liter of medium per bottle. Harvesting was on days 4 and 7 after virus inoculation. Medium without serum was used for the second harvest.

The harvests were passed through a 0.22-μm membrane filter (Millipore Corp.). To one-half of each harvest, 20% NZ-Amine AS (Sheffield Chemical Co.) was added to equal 3% of the final product as a bulking agent. Clear-glass, rubber-stoppered, aluminum-capped, 10-ml sterile ampoules each were filled with 5.5 ml of the virus suspensions. The groups with added bulking agent were lyophilized for 120 hr.

Determinations of viability were made by first diluting the virus suspension in 10-fold increments. Several dilutions, usually 10^-4 through 10^-7, were inoculated into Leighton tubes containing feline kidney cells and medium. Inoculum size was 0.1 ml per tube, and at least two tubes were used for each dilution. After 4 days of incubation at 37°C, the cover slips were removed from the Leighton tubes, and the viable virus was measured by using fluorescent-antibody techniques.

Ampoules were stored at 4°C, 25°C, and 37°C. The results of the long-range stability testing, shown in Table 1, demonstrate that both liquid and lyophilized vaccine are quite viable over a period of 13 months. The stability of the fluid vaccine is not due to serum in the medium.

Table 1. Stability of panleucopenia virus over a 13-month period

| Harvest | Storage temp (°C) | Titer (Log10 TCID50/ml) |
|---------|-----------------|-----------------------|
|         | Orig. | Day 50 | Day 60 | Day 80 | Day 180 | Month 13 |
| Liquid: |       |       |       |       |         |         |
| I       | 5     | 7.0   | 7.5   | 7.5   | 7.5     | 7.5      |
| I       | 25    | 7.0   | 7.5   | 7.5   | 6.5     | 7.0      |
| I       | 37    | 7.0   | 7.5   | 6.5   | 4.0     | NTa      |
| II      | 4     | 6.5   | 7.0   | 6.5   | 7.0     | 6.5      |
| II      | 25    | 6.5   | 6.5   | 7.0   | 7.0     | 6.0      |
| II      | 37    | 6.5   | 6.5   | 6.0   | 4.0     | NT       |
| Lyophilized: | |       |       |       |         |         |
| I       | 4     | 7.0   | 7.5   | 7.5   | NT      | 7.5      |
| I       | 25    | 7.5   | 7.0   | 7.5   | NT      | 7.0      |
| I       | 37    | 7.0   | 6.5   | 7.0   | NT      | NT       |
| II      | 4     | 6.0   | 7.0   | 7.0   | NT      | 7.0      |
| II      | 25    | 7.0   | 6.5   | 7.5   | NT      | 6.5      |
| II      | 37    | 6.5   | 6.5   | 6.5   | NT      | NT       |

*aNT = Not tested.
dium because the second harvest had no serum in the medium. Virus in liquid at 37 C showed a definite loss of viability at 180 days.

Exposing vials of the liquid suspension to 56 C in a water bath for 1 hr and then to direct sunlight for 8 hr did not alter the virus titer.

In view of the data, the suggestion of a live, modified virus vaccine in liquid form seems plausible and would obviate lyophilization and reconstitution of the vaccine. However, antigenicity of the liquid vaccine after the 13-month storage period still needs to be verified.

LITERATURE CITED
1. Andrewes, C., and H. G. Pereira. 1967. Viruses of vertebrates, 2nd ed. The Williams & Wilkins Co., Baltimore.
2. Scott, F. W., C. K. Csiza, and J. H. Gillespie. 1970. Feline viruses. IV. Isolation and characterization of feline panleukopenia virus in tissue culture and comparison of cytopathogenicity with feline picornavirus, herpesvirus, and reovirus. Cornell Vet. 60:165-183.