PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 152, 554-556 (1976)

Stability of Rauscher Leukemia Virus under Certain Laboratory Conditions (39438)

HAROLD B. STULL AND ADI F. GAZDAR

Laboratory of Viral Carcinogenesis, National Cancer Institute, Bethesda, Maryland 20014

Type C viruses are commonly encountered laboratory agents. Several type C viruses, including known oncogenic strains, replicate efficiently in human cells, representing biohazards of unknown potential. Virus mutants, conditionally or nonconditionally defective in replication, and viral recombinants, created deliberately or inadvertantly, are also potentially biohazardous (1). The National Cancer Institute (NCI) has formulated guidelines for the laboratory handling of biohazardous agents (2), but relatively little is known about the survival of type C viruses under laboratory encountered conditions. In this report we describe survival studies on Rauscher leukemia virus (RLV) (3) which may assist the further development of safe laboratory handling procedures.

Materials and methods. Biological laminar cabinet. All cell culture and virus procedures were performed in a 4-ft laminar flow biological safety cabinet class II (BioQuest) with an average vertical air flow velocity of 48 ft/min. Thermal temperatures of the cabinet work surface were initially ambient (25°), gradually increased to 28–29° after 3 hr of operation, and then remained steady. A new ultraviolet (uv) lamp (GE-G36T6) was installed into the provided cabinet fixture and activated 24 hr prior to use. The dose rate of the uv lamp was 500 $\mu W/cm^2$ at the work surface distance of 54 cm.

Virus. RLV stock was obtained from a chronically infected JLS-V9 subline (4) obtained from Dr. G. Shibley. The cells were grown in RPMI 1640 medium supplemented with 10% heat inactivated fetal bovine serum (FBS) and antibiotics. Supernatant fluids were harvested at 24-hr intervals, clarified by centrifugation (4°, 12,000g, 10 min). Pooled samples were millipore filtered (0.45 μm), aliquoted, and stored at −70°. The virus was quick-thawed prior to use.

Virus assay. RLV was assayed by a direct focus assay. A1–2 cells are a clone of sarcoma virus positive, helper virus negative (S+H−)-transformed BALB/c peritoneal (BP) cells (5). Superinfection with B- or NB-tropic viruses results in the rapid development of foci of morphologically altered cells, similar to the S+L-assay of Bassin, *et al.* (6). Indicator cells were plated at a density of 1×10^5/60 mm plate and duplicate plates infected (1 hr, 37°) with 0.3–0.5 ml of virus dilution 24 hr later. Infected plates were fed with 7 ml of growth medium (Eagle's minimal medium supplemented with 10% heat-inactivated FBS and antibiotics). Foci were enumerated 5 days after infection.

Drying method and uv inactivation. Virus samples were dried by spreading (glass spreader 1 × 20 mm) undiluted virus (0.1 ml) over an area of 64 cm^2 on 150-mm integrid plastic plates (Falcon) and exposed to the air flow of the safety cabinet. Since preliminary experiments indicated that complete drying was achieved in 8–10 min, virus survival studies were performed on samples allowed to dry for 10 min. At various times after drying, 0.5–1.0 ml of cold medium was added, and the virus reconstituted by scrubbing with the glass spreader. This process was repeated, and the two samples were pooled and assayed immediately.

Ultraviolet inactivation was determined by exposing plates of dried virus immediately after drying to the uv lamp installed in the cabinet at a distance of 54 cm, and the virus reconstituted as described above.

Polymerase assay. RNA-dependent DNA polymerase (RDDP) activity was determined by a previously published method (7). Virus fluids were concentrated (4°, 100,000g, 1 hr) in a Beckman SW41 rotor and assayed for RDDP activity using the synthetic template $(dt)_{12-18} \cdot (rA)_n$ and results are expressed as Δ-pmoles of $[^3H]$dTMP product/ml of unconcentrated virus after 60 min of incubation at 37° (1 pmol = 19,422 cpm).

Results. The results presented represent

Copyright © 1976 by the Society for Experimental Biology and Medicine
All rights reserved.

the mean values of two to three experiments, and unless otherwise stated, were obtained using a single serum lot.

The thermal stability of RLV was tested at refrigeration (4°), ambient (25°), and incubator (36°) temperatures, and the data presented in Figs. 1 and 2 and Table I. Infectivity and RDDP activity were most stable at 4° and least stable at 36° (Table I). At all temperatures tested, RDDP activity was more stable than infectivity. This is demonstrated in Fig. 2 by the decrease with time of the number of FFU/pmol of RDDP activity.

The infectivity of virus suspended in serum-free medium decreased more rapidly than virus suspended in medium supplemented with 10% FBS. Serum lots varied considerably in their stabilizing effect, and the data presented were obtained with the optimal serum lots tested.

The survival of dried RLV was investigated (Fig. 3). A thin film of virus was allowed to dry in the safety cabinet for 10 min. Drying of the virus resulted in a tenfold reduction in titer. A further tenfold reduction in titer occurred during the first hour after drying. Subsequently, inactivation proceeded at a reduced rate, with an approximate half-life of 1 hr. When ethanol (70%) was sprayed onto the dried virus and permitted to evaporate prior to reconstitution, viable virus was not detected.

Figure 4 shows the inactivation with uv

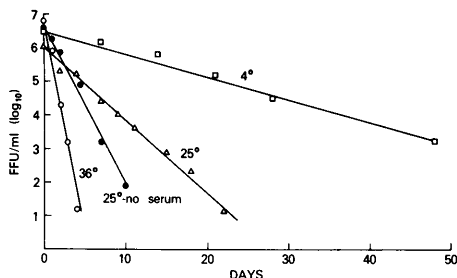

FIG. 1. Thermal stability of RLV. See Table I. Fetal bovine serum was omitted from the diluent of the specimen incubated at 25° and labeled "no serum."

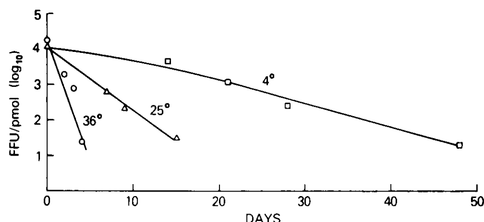

FIG. 2. Differential survival of RLV infectivity and RDDP activity. See Table I. The ratio of virus infectivity (FFU) to RDDP activity (pmol) is plotted as a function of time.

TABLE I. SURVIVAL OF RLV INFECTIVITY AND RDDP ACTIVITY.[1]

	Half-life	
Incubation temperature	Focus forming activity	RDDP activity
4	7 days	25 days
25	36 hr	10 days
36	7 hr	13 hr

[1] Stock RLV was diluted in growth medium and incubated in a refrigerator (4°) or water jacketed incubators (25° and 36°). Aliquots were periodically removed and tested for infectivity and RDDP activity. The figures represent mean values of two to three experiments.

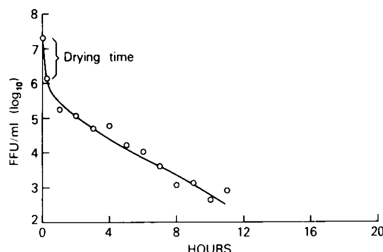

FIG. 3. Stability of air dried RLV in growth medium at 28–29° (Laminar cabinet temperature). Virus samples were spread on petri plates and air-dried in a safety cabinet under standard conditions, as described under material and methods. Samples on individual plates were reconstituted periodically and immediately assayed for surviving infectivity.

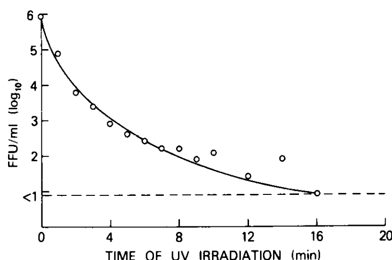

FIG. 4. UV inactivation of dried RLV. Virus samples were spread on petri dishes, air-dried in a safety cabinet (see materials and methods), and exposed to the uv light source installed in the cabinet (500 μW/cm^2). Samples were periodically reconstituted and assayed immediately for surviving infectivity.

irradiation of air dried RLV using the light source installed in the safety cabinet. Sixteen minutes of irradiation inactivated over 5 logs of virus.

Discussion. Our results indicate that the half-lives of RLV at refrigeration, ambient, and incubator temperatures are 168, 36, and 7 hr, respectively. Virus suspended in FBS-supplemented medium survived longer than virus suspended in protein-free medium. These results are in general agreement with those of Levy *et al.* (8) for the survival of RLV oncogenicity, and of Smith (9) for the survival of Rous sarcoma virus infectivity. RDDP activity was more stable than focus-forming ability at all temperatures tested. The greater stability of RDDP activity should be considered whenever it is used as an indicator of infectivity.

After the first hour, air-dried virus had a half-life of approximately 1 hr. Exposure of dried virus to the uv source installed in the safety cabinet inactivated more than 5 logs in 16 min. Dried virus was inactivated also by spraying with 70% ethanol. The relative stability of dried virus and the known aerosol stability (10) and spread of RLV induced leukemia (11) increase the potential biohazard of type C viruses.

These studies indicate that RLV is relatively stable under a variety of laboratory encountered conditions, providing opportunities for accidental exposure of laboratory personnel, as well as inadvertant contamination of other virus stocks and cell cultures. Recommended laboratory procedures include the use of laminar flow safety cabinets, decontamination of the work surface and interior wall of the cabinet with ethanol, or other chemical agents, and exposure of the cabinet interior to uv light for at least 15 min between handling different cell cultures or viruses.

Summary. The stability of Rauscher leukemia virus (RLV) was investigated under certain laboratory conditions. The half life of the virus at 37° was 7 hr, and considerably longer at lower temperatures. RNA dependent DNA polymerase activity was more stable than infectivity at all temperatures. Air dried virus had a half life of approximately 1 hr, but was rapidly inactivated by uv light or 70% alcohol.

We are indebted to Drs. W. Emmett Barkley and Herbert K. Oie for advice and encouragement, and Edward Russell for technical assistance.

1. Weiss, R., Nature (London) **255,** 455 (1975).
2. DHEW Publication No. (NIH) 75-790 (1974).
3. Rauscher, F. J., J. Nat. Cancer Inst. **29,** 515 (1962).
4. Wright, B. S., O'Brien, P. A., Shibley, G. P., Mayyasi, S. A., and Lasfargues, J. C., Cancer Res. **27,** 1672 (1967).
5. Gazdar, A. F., Russell, E. K., and Minna, J. D., Proc. Soc. Exp. Biol. Med. **149,** 688 (1975).
6. Bassin, R. H., Tuttle, N., and Fischinger, P. J., Nature (London) **229,** 564 (1971).
7. Gazdar, A. F., Russell, E. K., and Minna, J. D., Proc. Nat. Acad. Sci. USA **71,** 2642 (1974).
8. Levy, J. P., Oppenheim, S., Chenaille, P. H., Silvestre, D., Tavitian, A., and Boiron, M., J. Nat. Cancer Inst. **38,** 553 (1967).
9. Smith, P. E., Virol. **60,** 543 (1974).
10. Larson, E. W., Spahn, G. J., Peters, R. L., and Huebner, R. J., J. Nat. Cancer Inst. **44,** 937 (1970).
11. McKissic, G. E., Griesemer, R. A., Farrell, R. L., J. Nat. Cancer Inst. **45,** 625 (1970).

Received December 15, 1975. P.S.E.B.M. 1976, Vol. 152.