

β -Propiolactone Decontamination of Simian Virus-40 as Determined by a Rapid Fluorescent-Antibody Assay

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ABSTRACT

LEVINE, S. I. (The National Drug Co., Philadelphia, Pa.), N. R. GOULET, AND O. C. LIU. β -Propiolactone decontamination of Simian virus-40 as determined by a rapid fluorescent-antibody assay. *Appl. Microbiol.* **13**:70-72. 1965.— β -Propiolactone vapor treatment of vaccine production facilities has been shown to be approximately 90% effective in the elimination of large quantities of Simian virus-40 (SV₄₀). The use of a rapid fluorescent-antibody assay for the detection of SV₄₀ was also studied.

Simian virus-40 (SV₄₀) contamination of animal quarters and of production or research laboratories is of considerable concern to manufacturers of virus vaccines. β -Propiolactone (BPL) vapor has been utilized to decontaminate enclosed spaces (Bruch, 1961; Hoffman and Warshowsky, 1958). The efficacy of BPL decontamination in these studies was based solely on results obtained with exposed bacterial spore strips.

This report is concerned with BPL vapor decontamination of a specific virus (SV-40) intentionally introduced into three vaccine production areas, and the development of a rapid assay for the detection of SV₄₀.

MATERIALS AND METHODS

Virus. SV₄₀ used was isolated from a formalinized adenovirus vaccine. The virus was propagated in African Grivet monkey kidney (AGMK) cultures. The infectious titer of the virus was approximately $10^{8.0}$ per ml. Identification was established by cross-neutralization tests employing rabbit antisera prepared against the homologous virus and the 45-54 strain of SV₄₀ (obtained from G. W. Workman, National Institutes of Health).

Decontamination Procedure. Three buildings were included in the experiment: an animal holding barn, a vaccine production building, and a research facility. A large quantity of SV₄₀ ($10^{-1.0}$ dilution) was swabbed on the floors, bench tops, sinks, cupboards, etc., in wax-delineated areas. As a control against inactivation of the virus by residual surface disinfectants, these areas were sampled for SV₄₀ 1 day later by reswabbing with a sterile moistened swab, and then retreated with fresh virus. Decontamination (performed by the

Wilmot Castle Co., Rochester, N.Y.) of each area was effected by atomizing a quantity of BPL calculated to give a maximal concentration of 10 mg per liter of space. The temperature and relative humidity were kept to 30 C and 80%, respectively. Since the half-life of BPL at 25 C is 210 min (Hoffman and Warshowsky, 1958), it was deemed safe to work in the treated areas after a minimum of 4 hr. However, as an added precaution, the treated areas were kept sealed overnight. Although the BPL should have been completely hydrolyzed in that time, a residual odor remained. This odor was dissipated after an aeration period of 15 min. The wax-delineated areas were then sampled for a second time. Swabs were placed in a tube containing sterile saline (Hanks and Wallace, 1949), 400 units of penicillin G, and 1,000 mg of streptomycin, and held at -40 C until assayed for SV₄₀.

The effectiveness of the decontamination procedure *per se* was determined by placing duplicate spore strips of *Bacillus subtilis* var. *niger* (*B. globigii*) or *B. stearothermophilus* in open petri dishes at strategic locations in each building. The petri dishes were sealed after BPL decontamination, and the spore strips were tested (performed by the Wilmot Castle Co., Rochester, N.Y.) for the presence of viable bacteria.

Detection of SV₄₀ by the fluorescent-antibody technique (FA). Rabbit antiserum prepared against SV₄₀ was fractionated with ammonium sulfate and conjugated with fluorescein isothiocyanate. The conjugated globulin was then dialyzed against a 0.01 M, pH 7.2 phosphate-buffered saline (PBS) at 4 C until free of visible fluorescence. The conjugate was adsorbed twice with mouse liver powder to remove nonspecific substances.

Cover slip AGMK cultures were inoculated with 0.5 ml of the samples to be assayed and incubated

at 37 C for 7 days. The cultures were then washed four times with PBS, air-dried, and fixed in acetone for 10 min. Fluorescent staining was done in a moist chamber by covering the cover slip cultures with the conjugated globulin and incubating at 37 C for 60 min. Cultures were washed twice in PBS, once in distilled water, air-dried, and mounted on a microslide (Rodriguez and Deinhardt, 1960). The cultures were then read for SV₄₀ nuclear fluorescence.

Comparison of the FA technique and cytopathogenic effect (CPE) method. A direct comparison of the FA and CPE methods of detecting SV₄₀ was made as follows. For the FA method, 13 AGMK slide cultures were challenged with 0.1 ml of SV₄₀ for each half-log virus dilution through 10^{-9.5}. The cultures were incubated at 37 C for 7 days and then stained for nuclear fluorescence as described above. For the CPE method, 15 AGMK cultures were challenged with 0.1 ml of the same virus dilutions. Cultures were read for characteristic SV₄₀ CPE for 25 days. Those cultures not showing CPE were individually harvested, frozen and thawed once, subpassed into each of two fresh AGMK cultures, and observed for another 20 days.

RESULTS

BPL decontamination of the spore-containing strips was shown to be 99.99% effective. This figure was obtained by comparing the number of bacteria surviving BPL treatment with the number of bacteria originally present.

Table 1 summarizes the data obtained with SV₄₀. Of the 25 pairs of swabs taken, 16, or 88%, were SV₄₀-positive before BPL treatment, and SV₄₀ negative after BPL treatment. Two pairs of swabs, or 11%, showed evidence of SV₄₀ in both pre- and post-BPL samples. Seven pairs of swabs were not amenable to testing because of bacterial contamination or negative SV₄₀ findings in predecontamination control swabs. In six instances (33%), SV₄₀ was detected by positive fluorescence in spite of negative CPE.

Results obtained in comparing the FA and CPE methods employed for the detection of SV₄₀ are presented in Fig. 1. It can be seen that, after 7 days of incubation, the FA method was approximately 10-fold more sensitive than the CPE method. At the end of 14 days of incu-

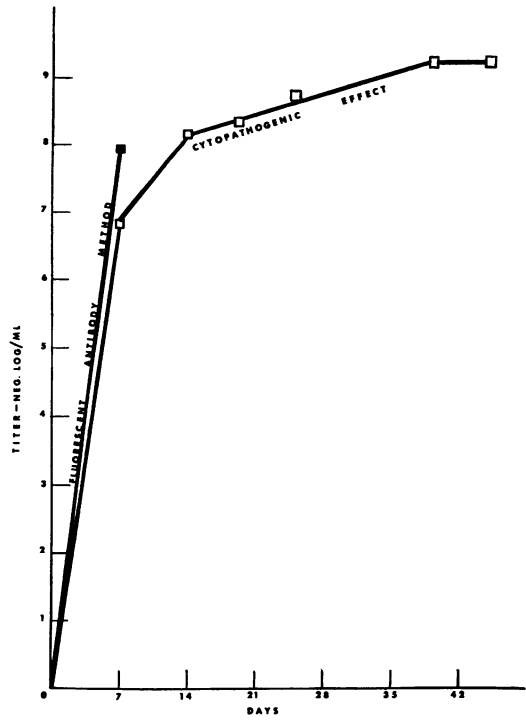


FIG. 1. Titer of SV₄₀ assayed by the fluorescent-antibody and cytopathogenic effect methods.

bation, the sensitivity of these methods was comparable. Beyond this, the titer obtained by the CPE method (including one subpassage) increased until 39 days. At this time, and at 45 days, the CPE method was approximately 20-fold more sensitive than the FA method.

DISCUSSION

It is known that SV₄₀ can be isolated from monkey kidney tissue cultures (Sweet and Hilleman, 1960) and that formaldehyde does not completely inactivate this virus in vitro (Gerber et al., 1961; Randall et al., 1963). Evidence in our laboratory suggests that housing of SV₄₀-free monkeys in an area where SV₄₀ was known to be harbored may eventually lead to infection of all monkeys.

Results presented here demonstrate that BPL treatment of monkey holding quarters or research laboratories, under controlled atmospheric conditions that are known to kill bacterial spores, can significantly reduce the level of contamination by SV₄₀. The efficacy of SV₄₀ decontamination by BPL might have been greater had smaller quantities of SV₄₀ been introduced, i.e., it is reasonable to assume that the natural SV₄₀

TABLE 1. Assay results on SV₄₀ before and after decontamination

Virus	Controls		Post-BPL vapor	
		%		%
SV ₄₀ -positive	18/18*	100.0	2/18	11.2
SV ₄₀ -negative	0/18	0.0	16/18	88.8

* Positive or negative results/total assayed.

contamination of an area is not as extensive as that purposely established in this experiment.

Following the BPL decontamination of enclosed areas, caution should be exercised to determine whether the BPL has been totally hydrolyzed. Any quantity of BPL greater than 0.05 mg per liter of space can be detected by nasal irritation and lachrymation of the eyes (Hoffman and Warshowsky, 1958). A residual BPL odor can be dissipated by a short period of aeration before resuming the normal activity (housing of monkeys, propagation of tissue cultures, etc.) in the treated areas.

Although the FA technique has been used to detect SV₄₀ (Levinthal and Schein, 1963) the sensitivity of the method was not established. It has been demonstrated that the FA method did permit reasonably accurate detection of SV₄₀ in cultures after 7 days of incubation. The FA method can thus be used to screen rapidly a large number of virus samples to select those preparations requiring more critical examination for the presence of SV₄₀. Because of the retrospective nature of the CPE method, the FA technique has also been of value in screening cultures before use, either for viral propagation or for the safety testing of finished vaccines.

The data also confirm the belief that complete assurance of the absence of SV₄₀ can only be achieved by prolonged observation, which may involve several subpassages of the cultures.

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