APPLIED MICROBIOLOGY, Oct. 1968, p. 1465-1467
Copyright © 1968 American Society for Microbiology
Vol. 16, No. 10
Printed in U.S.A.

Evaluation of a Commercial Air Filter for Removal of Viruses from the Air

P. ROELANTS, B. BOON, AND W. LHOEST
Recherche et Industrie Thérapeutiques, R.I.T., Rixensart, Belgium

Received for publication 14 June 1968

The effectiveness of a commercial absolute air filter for removal of viruses from air was tested with an actinophage, under the usual conditions of a laminar airflow clean room. A new method of dry phage dispersion is described. The filter showed an average reduction of 99.996% of airborne actinophage.

Filtration is a very common technique for eliminating microorganisms from air. For several years, high-efficiency particulate air filters (HEPA filters or absolute filters) have been commercially available, and their capacity to retain bacteria is well known. The abundant information on this subject mainly concerns tests with spore aerosols within the 1- to 5-μm particle-size range. However, there is much less information available on virus particle filtration. The air contamination system is very critical in assays for filter efficiency with viruses. Since bacteriophage aerosols have been used most often, it seems likely that phage penetration into the filter was correlated with the droplet size. Washam (5) and more recently Jensen (3) tested HEPA filters with T-phage aerosols of *Escherichia coli* B having a droplet-size range higher than 1 μm. Harstad (2) obtained a submicron aerosol of *E. coli* B phage T1 by adding dextran to enhance the biological activity.

This communication reports the effectiveness of a commercial absolute filter for eliminating virus from air in the usual conditions of a laminar airflow clean room (1). The test was carried out with an actinophage which is more stable than the T phages of *E. coli*, thus allowing the phages to be dispersed as a viable dry suspension of submicron particles.

MATERIALS AND METHODS

Filter media. The HEPA filter evaluated in this study was a Vokes 55 asbestos filter. It was 99.95% efficient on methylene blue or sodium flame test procedure and its dimensions were 609 × 609 × 298 mm (Vokes Ltd, Guildford, Surrey, England).

For reference, two dust-removing filters were tested: a polyamid Viledon P15/500 prefilter and an acetate Viledon AS/360 filter (Carl Freudenberg, Weinheim, West Germany).

Filter testing apparatus. A small-scale model of a horizontal laminar airflow clean room was constructed as illustrated in Fig. 1. This apparatus consisted of a plexiglass chamber (60 × 60 × 100 cm) closed at both ends by an air filter. The passage of air through the inlet filter established a laminar flow at a constant rate of 0.5 m/sec (controlled with an anemometer). A Viledon P15/500 filter closed the room at the air exhaust end.

Production of actinophage. The actinophage used was *Streptomyces virginiae* ATCC 13 161 S1 phage, isolated in this laboratory. The number of phage particles in the stock suspension were counted by the method described below. For phage production in liquid medium, flasks with 30 ml of inoculum broth (soya meal, 20 g; calcium carbonate, 3 g; dextrose, 25 g; distilled water, 1 liter adjusted at *p*H 7.5) were seeded with 5.10^8 spores of *S. virginiae*. After incubation at 23 C on a rotary shaker (amplitude, 2 cm; 250 rev/min) for 40 hr, flasks with 30 ml of A C broth (Difco) were inoculated with 3 ml of the inoculum broth. This new culture was immediately contaminated with 10^9 phage particles. After incubation at 23 C on a rotary shaker for 20 hr, this production culture was filtered through a 0.45-μm Millipore filter (Millipore Corp., Bedford, Mass.) to remove mycelium debris. The diameter of the phage, previously determined by electron microscopy, appeared to be 0.05 μm.

Air contamination with actinophage. In experiments with phage aerosols, the top of a 250-ml glass chromatography sprayer (Desaga, Heidelberg, Germany) was inserted into the postexhaust filter chamber. The air outlet duct may be directly connected to the inlet of the blower to obtain a closed circuit. Portions (0.1 ml) of the phage suspension were injected into the air flow every 30 sec over a period of 15 min.

In assays with dry phage particles, the air outlet duct was disconnected for open circuit work. The phage suspension was nebulized on no. 2 Whatman filter paper with a glass chromatography sprayer. After drying under vacuum, the paper was placed on the inlet "ear" of the blower and the phage was dispersed in the filter-testing apparatus by suction through the paper. The submicronic nature of the phage particles was demonstrated by the following experiment. A classical all-glass Millipore filter apparatus was equipped as follows. Its funnel was covered by a cap with an orifice 0.5 cm in diameter, where the dry

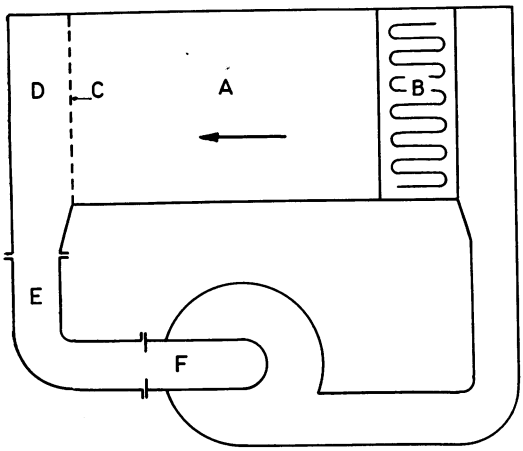

FIG. 1. *Filter-testing apparatus. (A) Plexiglass chamber; (B) tested filter; (C) outlet filter; (D) post-exhaust filter chamber; (E) air outlet duct; (F) inlet "ear" of the blower.*

filter paper saturated with actinophage was placed so that the air is sucked through the paper. When this apparatus was connected to a vacuum, the phage particles drawn into the air stream were recovered by impingement in a sampling solution (composition given below). The comparison between the phage recovery with and without a 0.45 μm membrane filter showed that the dry phage suspension contained more than 50% of particles less than 0.45 μm in size.

Actinophage sampling. Eight standard petri dishes containing 10 ml of the sampling solution (NaCl, 9 g; peptone, 2 g; Tween 20, 0.5 g; distilled water, 1,000 ml) were placed on the floor of the model clean room during the 15-min nebulizing period.

Counting procedure. A 10-ml amount of A C agar was poured into petri plates. Spores (5.10^6) of the sensitive *Streptomyces* were collected from a potato-glucose-agar slant mixed with 4 ml of melted A C agar at 45 C and poured on the base layer. After hardening, 0.3 ml of serial dilutions of the sampling solution were evenly distributed on the agar surface. This operation was repeated on five petri plates for each dilution of the sample. These dishes were then dried for 30 min at 37 C under vacuum and incubated for 40 hr at 25 C.

RESULTS AND DISCUSSION

The filter test apparatus employed in this study approximates the air filtering conditions usually found in laminar airflow clean rooms. Air contamination was obtained with actinophage aerosols on the one hand and dry actinophage submicron suspension on the other. Two dust filters and a commercial absolute air filter were compared. Tests with the Viledon P15/500 prefilter were used for reference. This type of filter has no capacity for phage retention but provides good laminar airflow conditions in the room (*see* Table 1).

The efficacy of the Viledon AS 360 filter or of the Vokes filter, expressed in per cent reduction, was determined by a comparison between the number of phages recovered in the clean room during assays with this filter and the phage recovery with the reference prefilter: per cent reduction = 100 − no. of phages recovered with test filter/no. of phages recovered with reference prefilter × 100.

These results compare favorably with those obtained by other authors. Harstad (2) found a reduction of 99.997% with a submicron aerosol, at an airflow rate of 0.3 m/sec, and Washam (5), with a microaerosol, measured a reduction of 99.999% at an airflow rate of 0.2 m/sec. Thorne (4) found a filter efficacy of 99.998% at an airflow rate of 0.1 m/sec using the foot-and-mouth disease virus.

TABLE 1. *Evaluation of a commercial air filter for virus elimination*

Mode of phage dispersion	Filter	Relative humidity	No. of phage particles collected in each assay[a]	Reduction
		%		%
Aerosol	None	65	3×10^3	
	Viledon P15/500	65	3.2×10^3	0
	Viledon AS/360	65	5.5×10^2	82
	Vokes 55	65	0.12	99.996
Aerosol	Viledon P15/500	80	8×10^2	
	Viledon AS/360	85	1.0×10^2	86
	Vokes 55	85	0	>99.99
Aerosol	Viledon P15/500	70	6×10^4	
	Vokes 55	70	1.2	99.998
Dry suspension	Viledon P15/500	60	1.25×10^3	
	Vokes 55	55	0	>99.99
Dry suspension	Viledon P15/500	50	3×10^4	
	Vokes 55	50	0.9	99.997

[a] Average value of phage titer in eight exposed petri plates.

It appears that an ordinary commercial HEPA filter (99.95% efficient on methylene blue or sodium flame test procedure) retains more than 99.996% of actinophage particles under laminar airflow clean room conditions, at an airflow rate of 0.5 m/sec, and that such a filter is suitable for virological work by using the laminar airflow principle.

LITERATURE CITED

1. Austin, P. R., and S. W. Timmerman. 1965. Design and operation of clean rooms. Business News Publishing Co., Detroit.
2. Harstad J. B., H. M. Decker, L. M. Buchanan, and M. E. Filler. 1967. Penetration of submicron T1 bacteriophage aerosols and bacterial aerosols through commercial air filters. 9th AEC Air Cleaning Conf. Proc., Boston, 13–16 Sept. 1966. **2**:701–714.
3. Jensen, M. M. 1967. Bacteriophage aerosol challenge of installed air contamination control systems. Appl. Microbiol. **15**:1447–1449.
4. Thorne H. V., and T. M. Burrows. 1960. Aerosol sampling methods for the virus of foot-and-mouth disease and the measurement of virus penetration through aerosol filters. J. Hyg. **58**: 409–417.
5. Washam C. J., C. H. Black, W. E. Sandine, and P. P. Elliker. 1966. Evaluation of filters for removal of bacteriophages from air. Appl. Microbiol. **14**:497–505.