Laboratory Equipment for Testing Filtering Materials for Air Sterilization

L. E. McDaniel and R. A. Long

Research Laboratories, Chemical Division, Merck & Co., Inc., Rahway, New Jersey

Received for publication March 23, 1954

In various operations connected with antibiotic production and other microbiological processes, large volumes of sterile air are required. One of the methods commonly employed for sterilizing air for such purposes is filtration through beds of materials such as cotton, carbon, slag wool or glass wool.

Preliminary to conducting pilot plant studies on design of air filters, some laboratory work was carried out to select the filtering materials which gave promise of being most effective and most adaptable to plant use. A description of the equipment used is given in this report as well as some examples of test results obtained.

Materials and Methods

Equipment

The procedure used was to contaminate a stream of air with bacteria, bacterial spores or bacteriophage and feed the air into two small filters arranged in parallel. Sampling devices were located before and after the filters so that "breakthrough" or presence of organisms in the air leaving the filters could be detected. Thus, a direct comparison of any two filtering materials could be made by packing them into the two parallel filters and determining the times required for breakthrough under identical conditions.

A diagram of the equipment used is given in figure 1.

Air is introduced from a compressed air line through a cotton filter contained in a glass tube (GF) and a flowmeter (Fl) into a Vaponephrin nebulizer (A-1) containing bacteria, spores or phage previously introduced through a port in the nebulizer. The contaminated air stream (A-1) is mixed with a secondary air supply introduced at A-2 to give any air flow desired above that required for nebulizing the culture. The contaminated air then enters a mixing chamber (MC) where the larger droplets settle out. The finely dispersed droplets pass on with the air to the test filters (TGF). Ahead of the filters is a mercury safety valve (M.SV) which releases excess pressure if it develops. Also, ahead of the filters is a sampler containing an impinger (1M-1) for determining the number of organisms in the air stream. The air divides between the two test filters (TGF), one of which holds a control filtering material and the other a test sample. From the filters, the air passes through air samplers containing impingers (1M-2 and 1M-3). The air then passes through flowmeters for measurement of the airflow through each filter. Finally the air is passed through cotton filters to trap any residual microorganisms and prevent gross contamination of laboratory air.

The air samplers are the capillary impinger type described by Rosebury (1947) and Kluyver and Visser (1950). They are 125-ml sidearm Erlenmeyer flasks joined to the apparatus with ground glass joints. The samplers hold 50 ml of sterile water. Air is introduced under the surface of the water through 1-millimeter capillary orifices. The side arms of the samplers are positioned so samples may be removed at intervals during the test runs. The samples are plated to determine the presence and the numbers of organisms.

The test filters are glass cylinders with ground glass removable ends. They are packed by introducing the sample filtering materials directly into the cylinders to the desired packing density. Suitable holders or clamps are used if necessary to hold the sample filtering material in place.

Operating Procedure

In most tests a well-sporulated culture of *Bacillus subtilis* morphotype *globigii* (*B. globigii*) was used as test organism. The culture was introduced into the air stream at a rate of 2.5 ml per hour which gave an air loading of around 20,000 cells per liter of air.

Air was passed through the nebulizer at 4 liters per minute. The secondary air rate was 0.5 liter per minute. The air rate through each of the samplers was 1.5 liters per minute.

The efficiency of the samplers in recovering organisms from an air stream was determined by using two samplers in series and finding the numbers trapped in each of the samplers by plate count. A second method was to pass the vented air from a sampler into a glass wool filter and determine the numbers of organisms caught in the sampler and in the glass wool filter. Some test results obtained by the latter method are given in table 1. By both test procedures, the

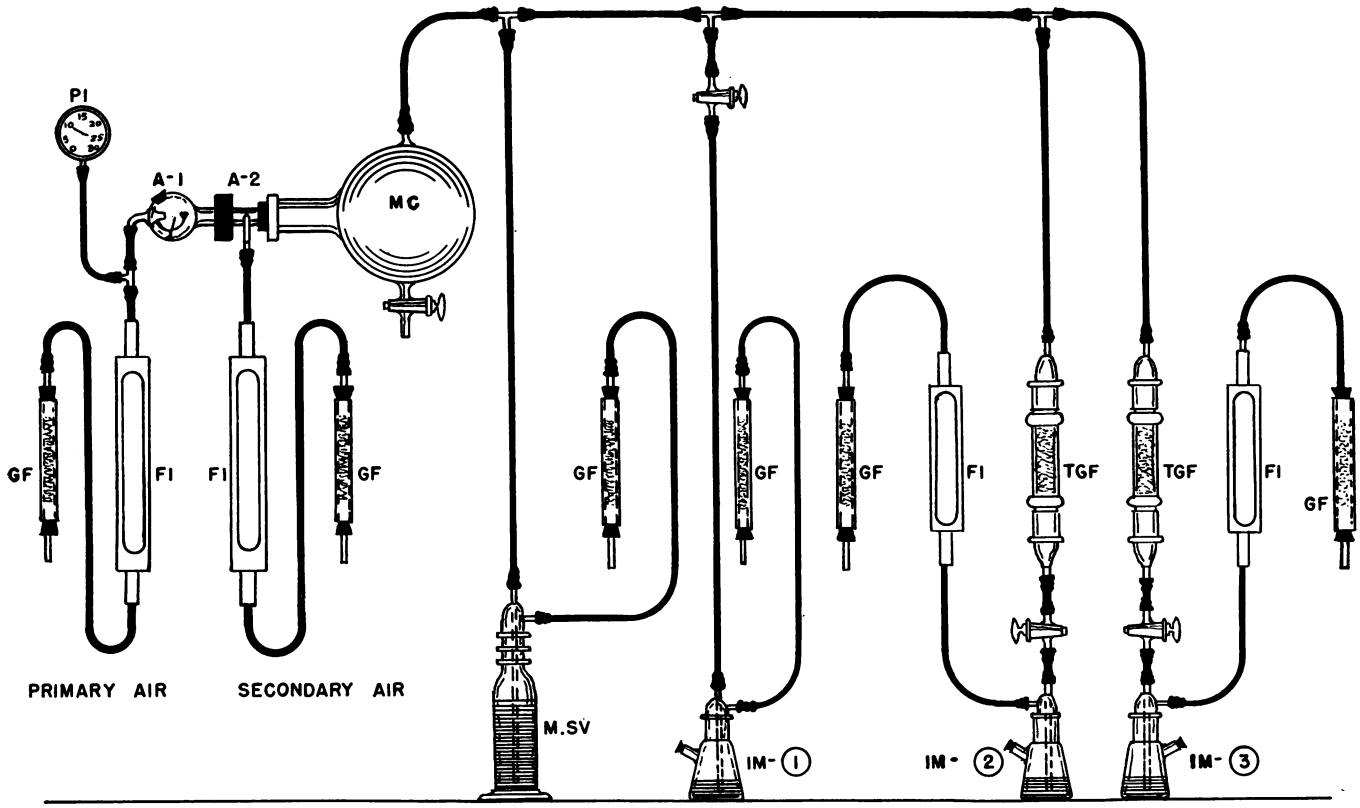

FIG. 1

samplers were found in all cases to be over 95 per cent efficient and in most cases over 99 per cent.

The recovery of organisms over the nebulizing system was determined by calculating the number entering the air stream by making plate count data of the suspensions used and determining the number of viable cells in the air stream by use of a sampler, IM-1, located just ahead of the filters. Table 2 shows the recovery of different organisms over this part of the system.

Nonabsorbent cotton was chosen as the standard filtering material against which other samples were compared. Six grams of cotton were packed into a 3-inch deep bed giving a density equivalent to 6.25 pounds per cubic foot. The linear air flow through the bed was approximately 6 feet per minute.

It was found that channelling was minimized and more reproducible breakthrough times were obtained when the cotton was packed as a series of pads rather than as one continuous packing. Therefore, six 1-gram pads of cotton were used in the control filter. The use of pads also made it easy to study penetration of the filter by testing for numbers of organisms trapped on each pad.

TABLE 1. *Recovery of microorganisms from an air stream by means of impinger samplers*

TEST ORGANISMS	RECOVERED IN SAMPLER	RECOVERED IN GLASS WOOL FILTER	RECOVERED IN SAMPLER AS PER CENT OF TOTAL RECOVERED
Bacillus globigii spores	7.5×10^5	102	99.99
Escherichia coli cells	8×10^9	500	99.99
Streptomyces griseus actinophage	1×10^7	1000	99.99

TABLE 2. *Recovery of different microorganisms over the nebulizing system*

TEST ORGANISMS	TOTAL NO. NEBULIZED	RECOVERED IN SAMPLER	PER CENT RECOVERED
Bacillus globigii spores	1.5×10^6	7.5×10^5	50
Escherichia coli cells	1.0×10^{11}	8.0×10^9	8
Streptomyces griseus actinophage	1.5×10^8	1.0×10^6	0.6

TABLE 3. *Test results with several filtering materials*

FILTERING MATERIAL	BED DEPTH	BREAK-THROUGH, HOURS
Cotton	3″	4
Carbon, 10 x 20 mesh	3″	2
Slag wool	3″	2
Glass wool	3″	3
Filter paper	1 sheet	3
Carbon, 30 x 50 mesh	3″	5
Asbestos-cellulose	1 sheet	>8

The filters were sterilized by autoclaving at 120 C for 1 hour.

Experimental Results

The results listed in table 3 illustrate how this equipment has been used.

Bulk materials were tested in volumes equal to those occupied by the cotton controls. Sheet materials were tested as single layers held between the ground glass joints.

The cotton controls gave an average breakthrough time of 4 hours. Slag wool and 10 × 20 mesh carbon were comparatively ineffective. Laboratory filter paper and 10 micron glass wool were somewhat better but inferior to cotton. 30 × 50 carbon was slightly better than cotton. A sheet of asbestos-cellulose paper[1] was the most efficient of the materials tested. In other experiments it showed no breakthrough in up to 190 hours. This material is that contained in the Cambridge Absolute Filter and is comprised of cellulose and asbestos fibers. (Northrup, 1953)

[1] Cambridge Corporation, Syracuse, N. Y.

Acknowledgment

We wish to acknowledge the receipt of the Cambridge Absolute Filter material from Mr. John Ayer of that corporation.

Summary

Laboratory equipment has been designed in which filtering materials may be tested for effectiveness for air sterilization.

Examples of filtration test results show that, in general, materials with the smallest particle size or smallest fiber size are more effective in removing bacteria and spores from air. An asbestos-cellulose filtering material was the most effective material tested.

REFERENCES

Kluyver, A. J., and Visser, J. 1950 The determination of microorganisms in air. Antonie van Leeuwenhoek. J. Microbiol. Serol., **16**, 299–310.

Northrup, David H. 1953 The A.E.C. or C.W.S. Air Filter. Chem. Eng. Progr., **49**, 513–517.

Rosebury, Theodor. 1947 *Experimental Air-Borne Infection.* The Williams & Wilkins Company, Baltimore, Md.