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Air Filters for Germ-free Isolators

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A germ-free isolator must have a perfect bacterial filter. This paper describes a new, relatively inexpensive, stainless-steel filter frame, which is easily and quickly assembled and protects the enclosed filter material at all times. Resistance to the flow of air was less than 4 inches of water at an airflow of 30 ft^3/min through the filter frame with 204 $inches^2$ of surface area and four, one-half inch thick pieces of fiberglass filter material. This filter performed satisfactorily in our gnotobiotic laboratory and was found to be consistently 100% efficient in removing an aerosol containing *Serratia marcescens* from an air stream under a variety of operating conditions.

Although air can be rendered free of bacteria in a variety of ways, filtration is probably the most commonly used method. Several authors have discussed air filtration in general terms (1, 2, 8), as well as its specific application to the isolation systems for the germfree animal (4, 6, 7). All investigators realize that a germ-free animal must constantly receive an adequate volume of bacteria-free air. However, the hot and humid isolator found frequently in an air-conditioned laboratory stands as mute testimony to the users' inadequate understanding of their air supply and filter systems.

Assuming that one must start with properly heated and humidified air, the quantity and quality of the air delivered to a germ-free isolator depends upon the air pump or blower, the type of filter material, and the filter frame. Satisfactory pumps are easily obtained, since various blowers and turbocompressors are available which allow the engineer to match the capacity and pressure of the pump to the airflow and back pressure characteristics of the filter system. Today, four, one-half inch thicknesses of fiberglass filter material wrapped on a perforated metal cylinder 3 inches in diameter by 12 inches long and covered by a plastic shroud, as described by Trexler and Reynolds (9), is the most commonly used filter for small isolators. From a practical standpoint, the ability of fiberglass filter material to produce bacteria-free air is unquestioned. However, there is relatively little published experimental data (3, 9) to support the efficiency of this filter material as it is presently used by those engaged in research with gnotobiotic animals. In addition, the investigator frequently does not appreciate the manner in which the resistance of the filter material affects the flow of air through the filter.

Despite the facts that the cylindrical filter frame is light, simple, relatively inexpensive, and can be readily designed with an increased diameter or length, or both, it has certain disadvantages. In our hands, it is somewhat difficult and time-consuming to perfectly wrap the filter material on the frame. Unless the cylinder is surrounded by a metal shroud (7), the filter material and its plastic cover are susceptible to external injury during storage and use after sterilization.

During the past 2 years, we designed and fabricated a set of filter frames, for use with sheets of fiberglass filter material, which eliminate these difficulties. This paper describes these filters, defines their resistance to airflow, and reports their efficiency as bacterial filters under operating conditions.

MATERIALS AND METHODS

Description of the filter frame. The stainless-steel filter frame is 24 inches high by 13 inches wide by 6 inches thick (Fig. 1). Two pans (no. 2002-1, The Vollrath Co., Sheboygan, Wis.), each 20.75 inches long by 12.75 inches wide by 2.5 inches deep, make up the basic structure. A piece of stainless-steel tube (16 gauge, 2 inches long by 2 inches outside diameter) is welded perpendicular to the middle of one of the 12.75-inch sides of one of the pans, thus forming an inlet to the filter. A tube of similar size is welded perpendicular to one end of the outer base of the other pan to form an outlet. A piece of expanded metal is welded to the inside of a 1-inch wide, 18-gauge rectangular frame, one side of which is continuously welded to the lip of each pan. An 18-gauge base is welded to the pan containing the outlet tube. Clips ("U"-shaped) along three sides hold the lips of the pans together and sandwich the filter material between the expanded metal covering the top of each pan. The surface area of the filter material is 204 $inches^2$.

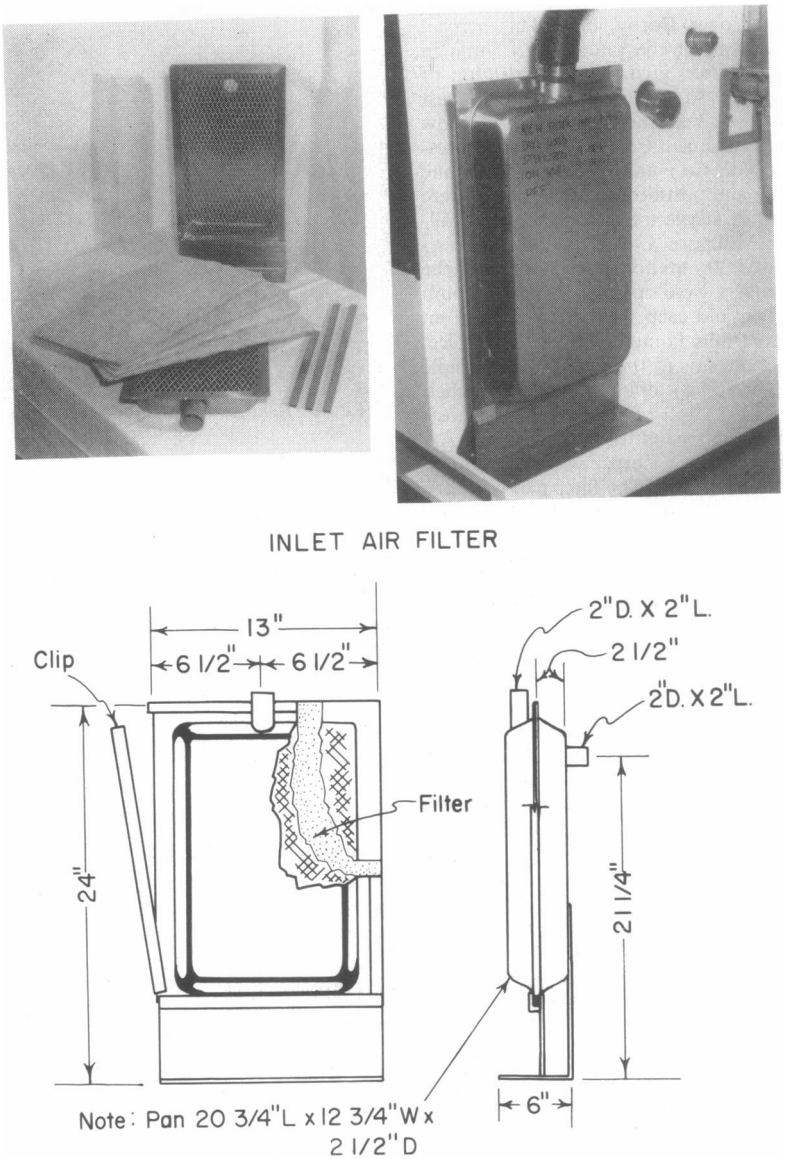

FIG. 1. *Lower, schematic drawing of the air filter; upper left, filter ready for assembly; upper right, assembled filter in place on a germ-free isolator.*

Filters of different sizes can be fabricated from pans of various sizes. Starting with two pans 16.38 inches long by 9.75 inches wide by 2.5 inches deep (Vollrath Co., no. 7416-2), a series of similar filters containing a surface area of 116 inches^2 were fabricated. These two sizes of filters and the common 100-inch^2 filter used in gnotobiotic laboratories are shown in Fig. 2.

Effect of flow rate on back pressure. Four, one-half inch thick pieces of FM-004 fiberglass filter material (Owens-Corning Fiberglas Corp., Toledo, Ohio) were placed in a series of the filters 116 and 204 inches^2. Using a turbocompressor (Spencer Turbine Co., Hartford, Conn.) and a laminar-flow element (Meriam Instrument Co., Cleveland, Ohio), the pressure on the inlet side of the filter was measured as the amount of air which was pushed through the filter was varied. The FM-004 filter material is composed of glass fibers with a nominal diameter of 0.00004 inch (1.02 μm) and has a surface density of 0.25 lb/ft^3.

Filtration efficiency. A suspension of *Serratia marcescens* containing 2×10^{11} cells/ml of sterile tryptose saline was prepared by the method of Decker et al. (1). A minimum of 0.2 ml/min of this suspension was atomized by directing an airflow of 6 liters/min at 15 lb/inches through a Vaponefrin nebulizer

(Vaponefrin Co., Upper Darby, Pa.). This aerosol was combined with air at the rate of 30 ft³/min in a 7.6-ft³ mixing chamber, giving a concentration of 5×10^8 cells per liter. After passing through the test filter, the air was sucked through a laminar-flow element (Meriam Instrument Co.) by a turbocompressor (Spencer Turbine Co.) and exhausted from the room. Prefiltration and postfiltration air samples were collected in 20 ml of sterile tryptose saline using all-glass impingers (Millipore Corp., Bedford, Mass.) with the stem raised 30 mm above the bottom of the flask. These impingers were operated with a vacuum of 20 inches of Hg, and each was found to have an airflow of 12.7 liters/min, limited by its critical orifice. The number of organisms in the prefiltration sample was determined by serial dilutions onto enriched nutrient-agar plates (1). All of the fluid in the postfiltration sample was passed through a type AA membrane filter (Millipore Corp.) with a pore size of 0.80 μm (Millipore Corp.). The filter pad was then placed onto an enriched nutrient-agar plate. All plates were incubated at 30 C for 24 hr. The percentage of filtration efficiency equals 100 times the number of organisms in the prefilter sample (minus the number in the postfilter sample) divided by the number in the prefilter sample.

Experiments. Four series of experiments were completed. In the first series, four new pieces of FM-004 fiberglass filter material (one-half inch thick) were placed in each of two filters and sterilized with dry heat at 151 C for 3 hr. *S. marcescens* was nebulized for 20 min and the prefilter and postfilter airflows were sampled for the last 15 min of the run. Six tests were completed at an airflow of 30 ft³/min and three at 15 ft³/min.

In the second series of experiments, two filters containing four pieces of FM-004 filter material (one-half inch thick), which had been used continuously to filter the air into a germ-free isolator at 35 ft³/min for a period of 3 months, were subjected to the same type of test used in the first series of experiments. Three tests were run at 30 ft³/min on each filter.

In the fourth series of experiments, a single filter with four new pieces of FM-004 filter material (one-half inch thick) was sterilized with dry heat. *S. marcescens* was continuously nebulized for 4 hr, and the pre- and postfilter airflows were sampled for 15 min during each hour.

In the fourth series of experiments, following a test procedure identical to that used in the first series, three separate Absolute filters (Model 1FS-25, Cambridge Filter Corp., Syracuse, N.Y.) containing a glass asbestos filter material with aluminum separators and a glass mat sealer in a cadmium plated steel frame were evaluated. Three tests were performed on each filter at 30 ft³/min.

FIG. 2. *The 204-inch² and 116-inch² surface area filters compared to the commonly used 100-inch² cylindrical filter with its plastic shroud.*

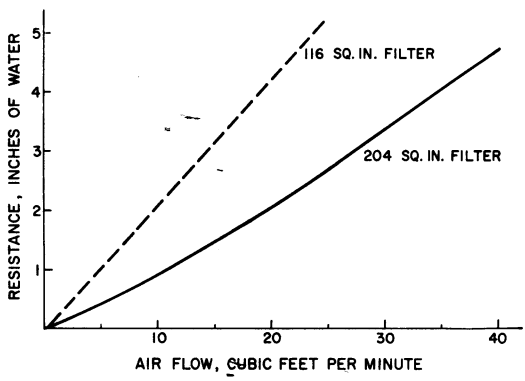

FIG. 3. *Relationship of filter resistance to airflow.*

RESULTS

Relationship of filter resistance to airflow. Figure 3 shows the relationship between the rate of airflow through the filter and the resistance (back pressure) to that flow by the filter. Note that the filter with a surface area of 204-inches² has approximately one-half the resistance to a given airflow as does the 116-inch² filter.

Filtration efficiency. During the past 2 years, the smaller filters (116 inches²) were used on a series of germ-free isolators at airflows of about 10 ft³/min, whereas the larger ones (204 inches²) were used at flow rates up to 40 ft³/min. None of our isolators has been contaminated as a result of filter failure. The temperature and relative humidity within the isolators were essentially that of the air supply (in most cases, the room air). From a practical standpoint, the filters produced bacteria-free air under working conditions.

During each of the 15 separate test runs of 20-min duration in the first and second series of experiments, approximately 8×10^{11} bacteria (*S. marcescens*) were nebulized and an average of 9.2×10^5 bacteria were found in the prefiltration samples. No bacteria were present in the postfiltration sample in any of these tests. After 3 months of continuous use, the filters had approximately the same resistance to flow as the new filter material, and they proved to be equally

effective in eliminating *S. marcescens* from the air stream.

During the 4-hr test in the third series of experiments about 10^{13} bacteria were nebulized and an average of 3.5×10^7 bacteria was found in the prefilter sample after each of the four, 15-min sample runs. Once again, no bacteria were found in any of the four 15-min postfilter samples. Therefore, this filter was 100% efficient.

During each of the nine, 20-min tests in the fourth series of experiments, about 8×10^{11} bacteria were nebulized and an average of 1.3×10^8 bacteria was found in the prefilter samples. In each test, bacteria were present in the postfilter samples and averaged 7.5×10^3 cells per sample. The filtration efficiency of these filters averaged 99.9994%. Thus, approximately one of every 20,000 bacteria in the air that entered the filter found its way through the filter.

DISCUSSION

Filtration efficiency test methods. The efficiency of a filter material may be determined by several nonbacterial and bacterial methods. Nonbacterial methods are commonly used and consist of some means of generating a series of small particles (e.g., dust, smoke (dioctyl-phthalate), and dye) and analyzing the postfilter air with a particle counter, photoelectric colorimeter, etc. Many of these methods are not sufficiently sensitive to evaluate a nearly 100% efficient filter. Although these methods can be used as a relatively gross screening test, they cannot be used to test filters for a germ-free isolator.

We believe that only biological methods can adequately test a bacterial filter. Various species of bacteria, phage, and viruses have been used as the biological test agents. In our experiments, a moderate size bacterium (*S. marcescens*), which measures 0.7 to 1.0 μm in length by 0.7 μm in diameter was nebulized by an atomizer which produces a high percentage of particles 1 μm in diameter (1). The high prefilter aerosol concentration of 5×10^8 cells per liter of air severely challenged the filters. The all-glass liquid impinger was chosen because of its high sampling efficiency, its high flow rate, and the ease with which it can be calibrated (5, 10). This bacteriological test method was very satisfactory.

Filter material. Air filters for germ-free isolators should be tested at the flow rates used under working conditions. Four thicknesses of FM-004 fiberglass filter material (each one-half inch thick) prevented any *S. marcescens* from penetrating the filter material under any of the test conditions. The filters were equally safe after 3 months of continuous use. These data, together with our experience with these filters on germ-free isolators, allow us to conclude that this filter material used in this manner is satisfactory for the gnotobiotic laboratory. It does not answer the question of whether three or possibly two thicknesses of the filter material would also be satisfactory. Undoubtedly, filtration of the air stream before it reaches the final filter on the germ-free isolator affects the safety one achieves by using a particular thickness of the filter material.

Other filter material may prove to be equally satisfactory. However, these experiments clearly demonstrate the danger of using a filter without biological testing. The Absolute ultrahigh efficiency filter (Cambridge Filter Corp.) is not efficient enough under some conditions to serve as a final filter for a germ-free isolator. Although our testing indicated that they were more efficient than claimed by the manufacturer, the filters did allow some bacteria to pass in every test

Air resistance. The resistance to the flow of air through a filter depends upon the filter material and the size of the filter frame. The commonly used 3-inch diameter cylindrical filter, with about 100 inches^2 of surface area, has a resistance of 1 inch of water at 5 ft^3/min and 4 inches at 15 ft^3/min. Our 204-inch^2 filter has a resistance of less than 4 inches at 30 ft^3/min. Once the investigator knows the resistance to the flow of air for his particular filter system, blower or turbocompressor of proper size can be identified and purchased.

Filter frame. The filter frame described in this paper effectively and consistently seals the filter material in place, as must all filters used for germ-free isolators. In addition, this filter has several other highly desirable features. The stainless-steel frame protects the filter material at all times after assembly and sterilization. The four flat pieces of fiberglass filter material are easily secured in the filter frame in less than 2 min. The assembled filter is ready for installation on the isolator immediately after sterilization without the additional time-consuming process of surrounding it with a plastic shroud.

This filter frame is somewhat heavier, larger, and costlier than the cylindrical filter with its plastic cover. However, the only significant disadvantage is the cost, which has been less than twice that of the cylindrical filter frame. In our view, the very significant advantages of this filter far outweigh this consideration.

ACKNOWLEDGMENT

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