

Advance Copy

QUALITY CONTROL
of
ABSOLUTE BACTERIA
REMOVAL FILTERS

by

David B. Pall Ph.D
Chairman of the Board
Pall Corporation



QUALITY CONTROL OF ABSOLUTE BACTERIA REMOVAL FILTERS

INTRODUCTION:

Achievement of bacteriological sterility requires that:

- A. The filter must reliably accomplish its removal function.
- B. The filter must not release into the downstream fluid significant quantities of foreign materials - either in solution or as solid particles.
- C. The filter is preferred to have long life, low pressure drop, ease of installation and sterilization, freedom from problems such as air binding, and low cost.

We shall concentrate in this paper on the means for assuring consistent quantitative removal of bacteria followed by a brief review of other quality control factors.

SIMULATED SERVICE TEST:

Duration of filtration operations varies from a few minutes up to several months or years of continuous or intermittent flow. In order to provide the highest possible reliability, while bearing in mind that a large number of tests must be run within a reasonable length of time, Pall's basic simulated service test has been set up as follows:

A suspension of the smallest commercially available bacterium, Pseudomonas diminuta (ATCC 19146), in water at $2 \text{ to } 4 \times 10^5$ per liter, is fed continuously at a rate of one liter/minute to the test cartridge over a period of 2 to 4 weeks, by which time the element may be clogged with bacteria to the extent that the one liter flow rate can not be maintained. Samples are taken daily and cultured; effluent

must be consistently sterile.

To supplement the above standard test, a smaller number have been run using higher bacteria loadings (hence less time to clogging and shorter test duration), and others have been run using lower bacteria loadings, with longer operating cycles.

NON-DESTRUCTIVE QUALITY CONTROL TEST:

The development of a satisfactory non-destructive test which correlates well with the above described simulated service test has occupied much of our energies during the last 18 months.

Consideration was given to the use of the "first bubble point" test. In this test, the filter is wetted, followed by measurement of the pressure required to make the first bubble appear. This test had its first industrial use to control quality of porous materials by Pall Corporation in the early 1950's. For many types of filters coarser than the bacteriological range it provides a very precise indication of maximum particle passed (1, 2). This same method is recommended by manufacturers of plastic membranes for use in the bacteriological range (3).

Two problems were experienced when we attempted to apply this test to Pall Corporation's new 0.2 μm rated AR element. First, when individual elements were wetted with water and subjected to visually observed bubble point testing, and the results correlated with the results of the simulated service test, it was found that a broad bubble point range existed, in which some elements would pass and others would fail the simulated service test; above

this range all elements passed, and below it all elements failed. Thus, a substantial proportion of the elements really suitable for service were being rejected, increasing our costs. Second, and more important, when a visually bubble free element was wetted and placed in a housing, and then pressurized to the same test pressure, a significant flow was measured at the outlet of the housing.

Our further investigations included the following observations:

- A. Pall Corporation bacterial filters are made of fibrous membrane. A widely used competitive filter medium, GS22, is of all plastic membrane construction. Using this plastic membrane we were unable under any circumstances to obtain zero flow in similar tests, even though applied pressures were less than one fourth those recommended by the manufacturers for testing. For example, with an assembly containing six square feet of GS22 membrane, which is recommended by its manufacturer to be tested at 40 psi, application of 10 psi resulted in a continuous flow of over 5 ml per minute of air.
- B. The same GS22 membrane was water wetted and tested as a flat disc in an open jig. While the normal first bubble point was 55 psi (3), observation at 10X with the aid of a stereo microscope showed a large number of bubbles, clinging to the membrane surface, after a 10 to 20 minute exposure to 10 psi of air pressure. This observation was quantified by measuring volume of air flow against time, with the results shown in Figure 7; the cause of the observed instability probably lies in the failure of water to wet the plastic of which the

C. When the same GS22 membrane was wetted with Varsol (a petroleum fraction with surface tension $1/3$ that of water), observation with a microscope at 10X magnification showed very active bubbling at 3 psi; in the Varsol these bubbles were continuously released from the filter surface. Based on the ratios of the surface tensions, this observation indicates a 9 psi or lower first bubble point in water (see infra equation (2) for relationship between bubble point, surface tension, and angle of contact.)

The behavior of the GS22 membrane described in paragraphs B and C was consistent in repeated tests with varying lot numbers, using specimens obtained directly from the manufacturer, as well as others supplied to us as a courtesy by a pharmaceutical manufacturing customer, who had in turn purchased them from the membrane manufacturer.

It was clear from the above that visual observations were difficult or misleading, due to the small size of the bubbles, or to the long time factors involved. It was reasoned that direct reading of flows at each pressure could be much more sensitive than visual observation, and in addition would be quantitative, easy to perform, and probably faster.

Apparatus was then built to permit quantitative measurement of flow vs pressure drop for various flat sheet materials. The flow detection device was a carefully calibrated Gilmont micro-flowmeter, which was used with a $1/20$ sq. ft. area flat specimen test jig providing a range from 8 ml/min/M^2 to 2000 ml/min/M^2 . Data obtained is shown in Figure 1, for GS22 and Pall Corporation's

AR grade, and for the nuclear type of membrane. The AR characteristics are plotted for single and double layers; the double layers are more significant because a double layer is used routinely in both disc and corrugated element applications. Run with Varsol, these tests were fast, and the resulting quantitative data consistently repeatable for a given specimen.

The following is an analysis of the theoretical significance of the curves of Figure 1.

Consider a filter consisting of a single capillary, of circular cross section, with diameter = D (Figure 2A.) The capillary is initially filled with fluid; the pressure P required to eject this fluid has been shown to be (4):

$$P = \frac{4\gamma}{D} \cos \Theta \quad (1)$$

where γ is the surface tension of the fluid, and Θ is the angle of contact between the liquid and the material of which the capillary is composed. If Θ is zero, $\cos \Theta = 1$, and the pressure required to eject liquids from a filled capillary is proportional to the surface tension. Comparing Varsol ($\gamma = 25.5$, $\Theta = 0$) to water ($\gamma = 74$, $\Theta = \text{finite}$) we see that the ratio

$$\frac{P_{H_2O}}{P_{Varsol}} < \frac{74}{25.5} \quad (2)$$

When the liquid wets the capillary surface, the angle of contact

Θ is zero, and we have

$$P = \frac{4\gamma}{D}$$

$$\text{or } D = \frac{4\gamma}{P} \quad (3)$$

Generally, the hole through a filter medium will not be a smooth circular capillary. Figures 4 and 5 show scanning electron micrographs of the AR fibrous membrane and of the GS22 plastic membrane media, which illustrate this convincingly. We must, therefore, direct our attention to openings of irregular cross sections, as illustrated in Figure 2B. Analysis of random shaped pores becomes mathematically difficult or impossible; however, it has been shown (2) that for a specific shape of pore wetted with a fluid of zero contact angle, a relationship exists between the diameter of the maximum spherical particle which will pass through it, and the pressure required to eject the fluid from the capillary

$$D = \frac{\text{Constant}}{P} \quad (4)$$

Real filters have numerous openings, not all of equal size, and are more nearly represented by Figure 2C. As pressure is increased, flow increases as each successively larger capillary opens up, and we should expect to generate a curve like that of Figure 1, in which the existence of flow at a given pressure drop corresponds to the existence of holes of a given size.

If we were to apply our pressure drop flow measurement test to the configurations of Figure 2C and Figure 2D, we should find that figuration 2D has more flow at a given pressure drop than Figure 2C; this reflects the larger number of large holes in 2D. Had we measured first bubble point only, we should have been misled into

assuming 2C and 2D to be equal, when in fact 2C is superior, having fewer large holes.

Some important conclusions may be drawn:

- A. Measurement of flow vs pressure drop yields more significant information than measurement of first bubble point only.
- B. Referring to the curves of Figure 1, the ordinate is a measure of the number of holes and the abscissa is in inverse relationship to the diameter of the holes.

Precise methods for computing the number of holes, and the actual pore diameters, appear not to be available. It is, however, possible to make some approximate computations, and this has been done as follows:

1. Known or estimated bacteria removal characteristics were used to convert the pressure scale to an "effective removal diameter" scale.
2. Conversion of the ordinate scale of Figure 1, from flow/unit area to number of holes per unit area can be accomplished as follows:

- (i) Assume that the filter medium consists of a series of parallel circular capillaries.
- (ii) Using Poiseuille's law, and the effective removal diameters derived in the preceding paragraph, calculate the number of capillaries corresponding to the measured flow data.

Step 1 involves assumptions which, if incorrect, could cause the pore diameter scale to shift, but the relative position of the various curves would remain unchanged. Step 2 appears justified by Jacobs (5), and is probably correct within a factor of four.

The resulting data is plotted for the largest holes, which account for 0.1% of the total flow, in Figure 3. We see that plastic membrane GS 22 has approximately 4×10^4 holes/cm² in the .21 to .22 μ m range, nearly zero holes in the .14 to .21 μ m range, and most of its holes in a narrow band below .13 μ m. Fibrous membrane AR shows a somewhat similar distribution range, with a spread approximately 0.02 μ m narrower than GS22, and with about 90% fewer openings on its high range of .21 μ m. Double layer of fibrous membrane AR has a still narrower pore size distribution range, and still fewer holes near its top limit of 0.2 micrometers.

At this point, we turn our attention to statistical considerations which indicate that in bacteriological filters, first bubble point is not a good criterion for gauging absolute removal. The total number of holes in both media is computed to be at least 6×10^{10} /cm² (if 0.1% of the flow is carried by 6×10^7 holes, then 100% of the flow is carried by no less than $1000 \times 6 \times 10^7$ holes), while the number of 0.22 μ m diameter bacteria collected to clogging is (by actual measurement) about 5×10^7 /cm². Consequently, the probability

of a slightly oversize hole being challenged by a bacterium is only about 1 in 1000.

We saw previously that the true first bubble point in 0.2 micrometer elements is very difficult to detect. We see now, that the true first bubble point is in addition not a good measure of element quality, since elements with a significant number of oversize holes can still provide a high degree of assurance of long term absolute bacteria removal. From this we may conclude that measurement of flow at a pressure corresponding to a pore diameter at the upper end of the permissible range will yield more significant data than seeking to measure the largest single hole.

It is popular to classify filters as "depth" as opposed to "surface" types. For many years, the plastic membrane types have been presented in the commercial literature (6) as surface types, while the Pall fibrous membranes have been called depth types. The inference has been that depth types have wide pore distribution, and surface types narrow. We see from Figure 3 that in fact the plastic membrane type has the wider pore size distribution; the AR grade is substantially more uniform than the GS22 grade, with respective relative spans of .135 to .21 compared with .125 to .22 μm . Realistically, discussion as to which is "surface" and which is "depth" type is idle; if the incident particle is 0.5 μm in diameter, both are surface filters; if it is .05 μm diameter, both behave as depth types.

A nearer approach to a true "surface" filter is the nuclear type, shown in Figure 6, made by etching openings through polycarbonate resin film; this type of element has a wider range than either the GS22 or AR, extending by the same criteria from under 0.1 μm to well over 0.22 μm . We see, then,

that the "surface filter" has the widest pore size range of the three, and can further conclude that there is no relationship between pore size distribution on the one hand, and whether a filter is of the so called "surface" or "depth" type on the other.

We indicated earlier that the units of the abscissa were derived from microbiological considerations. Briefly, these were:

Lower limit of GS22 was taken to be 0.13 micrometers, on the supposition that each grade will pass particles 1/2 its rated absolute diameter. This could just as well be taken as .16 or .18 micrometers.

Upper limit was taken as 0.22 micrometers because this grade quantitatively removes bacteria of this diameter; however, the statistical considerations noted above permit us to stipulate a small number (e.g. 100 to 1000 or more per cm^2) of large holes to exist in an effective absolute filter. On this basis, the largest pores in the non-nuclear membranes could be as large as 1.5 micrometers in diameter.

The possible existence of such wider ranges does not change any of the conclusions, and we may summarize this portion of the paper as follows:

- A. It can be shown statistically that first bubble point is not likely to be a good criterion for evaluation of the removal capability of bacterial filters.
- B. The plastic membranes now on the market have true first bubble points less than one-fourth of the advertised values; nevertheless,

these plastic membranes of the non nuclear type have been shown to function well for removal of bacteria; it appears that due to statistical nature of the process the small number of large holes present cause zero or an undetectably small number of failures.

- C. The fibrous membranes have narrower pore size distribution, when compared with non-fibrous plastic membranes and with the nuclear type.

The data of Figures 1 and 3 was obtained using Varsol, a refined petroleum fraction having a surface tension of 25.5 dynes/cm. Water as a wetting liquid was found unsuitable for the following reasons:

- (a) The non-fibrous plastic membranes contain a wetting agent, which reduces the surface tension to as low as about 45 dynes/cm; the degree of reduction of surface tension is variable, depending on how much has been washed through, etc.
- (b) Comparisons made using water are not valid, because neither fibrous nor plastic membranes have zero angle of contact with respect to water. Further, for both media flow of displacement air is time dependent, and no stable flow reading can be obtained. For example, when GS22 is water wetted and exposed to air differential pressure of 10 psi, flow rate gradually increases, reaching values in excess of 2000 ml/min/M² in one to two hours, as shown in Figure 7. By contrast flow of a Varsol wet membrane is stable with time.

TESTING OF PRODUCTION FILTER ELEMENTS:

For production filter element testing at Pall Corporation, the use of water is nearly mandatory; for example, in field operation when filtering aqueous fluids, the aqueous medium itself must be used. Since the fibrous membranes contain no wetting agent, and since our concern now is with the one type of material (AR-dual layer) only, we used water to develop the required quality control test.

The theoretical considerations reviewed above indicated that the test pressure should be equal to or slightly above the uppermost range of removal as recorded in Figure 1, which is 1.7 psi in Varsol. Taking into account the ratio of the surface tension of water to Varsol, a test pressure of 5.25 psi (10.6"Hg) was selected. At this pressure flow is quite stable, remaining constant for 16 hours or more.

A new type of test, known as a "Forward Flow Bubble Point" test, or more briefly as a "Forward Flow" test was developed. This test consists of wetting the element with water, subjecting it to a differential air pressure of 10.6"Hg in the normal flow direction, and measuring the volume of air flow. About 10 minutes is required for stable flow to be reached after air pressure is applied; the flow then remains stable for 16 hours or more.

The flowmeter used is capable of measuring air flows in the range from .04 to 15 ml/min. Area of a single cartridge is 5 sq.ft. ($.46M^2$) hence flows as low as .08 ml/min/ M^2 could be measured. We found that at 5.3 psid none of the elements showed lower flows than approximately .3 ml of air per minute, or .65 ml/min/ M^2 .

When an element is wetted by water, an aqueous membrane is formed which has an area of 5 sq. ft., and a thickness of only .006". An analysis of the cause of this minimum flow rate indicated that the observed volume of flow could be caused by the diffusion of air through the unimpaired membrane of water. This was confirmed to be the case by testing the same element at 75 °F using air and CO₂ alternately. Rate of flow of CO₂ was 48 times that with air. The diffusion coefficient of any non-reactive gas through membrane is proportional to its solubility, and the solubility of CO₂ in water at 75 °F is 48 times that of air; this coincidence demonstrates that flow of some 0.3 ml/minute through the 5 sq. ft. element is due to diffusion of air through the membrane of water.

Correlation between the forward flow test and the simulated bacteria removal service test was accomplished in the following manner. Elements were selected to cover a wide range of forward flows; these were tested by the forward flow test and then subjected to the previously described simulated service test. The results are shown in Table I. Note that no element with a flow of under 199 showed bacteria passage in less than 2 to 3 weeks of continuous operation. In order to provide a factor of safety, flow of 100 cc/hour has been established as the maximum flow for an acceptable production element. For shorter term tests, up to 96 hours, a considerably higher standard could be considered.

Statistically, the correlation between grow through results and forward flow bubble point data is 97%, which represents a substantial improvement over the 77% obtained using the first bubble point method.

FIELD TESTING PROCEDURES:

The "forward flow" test system adapts itself very well to in-plant testing by the purchaser. Such testing is done in either of the following ways:

- A. Where the element is removed from service, or is to be tested prior to service, the apparatus shown in Figure 8 is used. The element is water wetted, 10.6" of Hg vacuum applied for 10 or more minutes, and the resulting flow read. Apparatus for evacuating ten or twenty cartridges simultaneously is available.
- B. A second configuration, shown in Figure 9, is a conveniently arranged accurately pressure balanced pneumatic trough, used for "in-situ" testing of presterilized filter assemblies. Air pressure (5.25 psi) is applied for a 10' stabilization period to the inlet of the prewetted assembly, while the effluent air is directed by a 3-way valve to bubble through the liquid in the trough to atmosphere. The 3-way valve is then turned to direct flow to the calibrated burette for 30" or 60", and volume of flow read. Back contamination can be prevented by using alcohol in the trough.

OTHER QUALITY CONTROL PROCEDURES:

The AR filter medium is very well bonded, the volume of resin binder being 60% that of the fiber to be bonded. Repeated shock high pressure differential flow tests result in no release of solids from this fibrous membrane. Release of particles downstream of the element, due to "built in dirt" is kept to extremely low levels by observing clean manufacturing procedures, followed by a very high velocity water flush, which simultaneously exposes the cartridge to a differential pressure sufficient to

test of its integrity at high differential pressure. This is followed by the "forward flow" bubble point test, drying and packaging. Levels of downstream particle release compare more than favorably with comparable competitive plastic membrane types (8).

Organic contaminants in aqueous effluent are normally zero by the potassium permanganate test. Pressure drop and cleanliness are checked on an AQL basis. Element can be repeatedly steam sterilized with no loss of characteristics.

TABLE I

Correlation of Pseudomonas Grow Through Tests with Forward Flow
Bubble Point Tests

Element Serial Number	Forward air flow @ 10"Hg, water wet, cc/hour	Hours on stream at 1 L/min. and $2 \text{ to } 4 \times 10^5$ <u>Pseudomonas diminuta per minute</u>		
		Clogged, with zero grow through	Removed prior to clogging with zero grow through	Removed prior to clogging, due to grow through at time noted
F6	16	-	288	
H105	24		384	
J3	27		480	
I70	28		384	
PTM15	39		336	
J6	39		576	
2389N	62		384	
H72	81		480	
PTM14	111		336	
J79	112		384	
PTM2	120		336	
PTM11	123		456	
H97	125		432	
PTM24	153		456	
I68	190	336		
PTM20	198	360		
98-176	199			192
I53	210		456	
J194	213		360	
PTM21	213		456	
PTM23	216		338	
97-1	225			96
J4	230	480		
B49	238			96
90-23	238		312	
J23	258		456	
J79	268	648		
J75	270		240	
I67	282		264	
97-342	291			144
J-84	315	528		
98-65	361			96
PTM4	363		336	
J50	400	432		
H67	450		504	
PTM8	471			96
98-77	545			120
97-201	600			144
B37	645		100	

TABLE I (continued)

Correlation of Pseudomonas Grow Through Tests with Forward Flow
Bubble Point Tests

Element Serial Number	Forward air flow @ 10"Hg, water wet, cc/hour	Hours on stream at 1 L/min. and $2 \text{ to } 4 \times 10^5$ <u>Pseudomonas diminuta per minute</u>		
		Clogged, with zero grow through	Removed prior to clogging with zero grow through	Removed prior to clogging, due to grow through at time noted
J52	645		432	
J68	650	384		
I17	685		480	
97-222	900			1

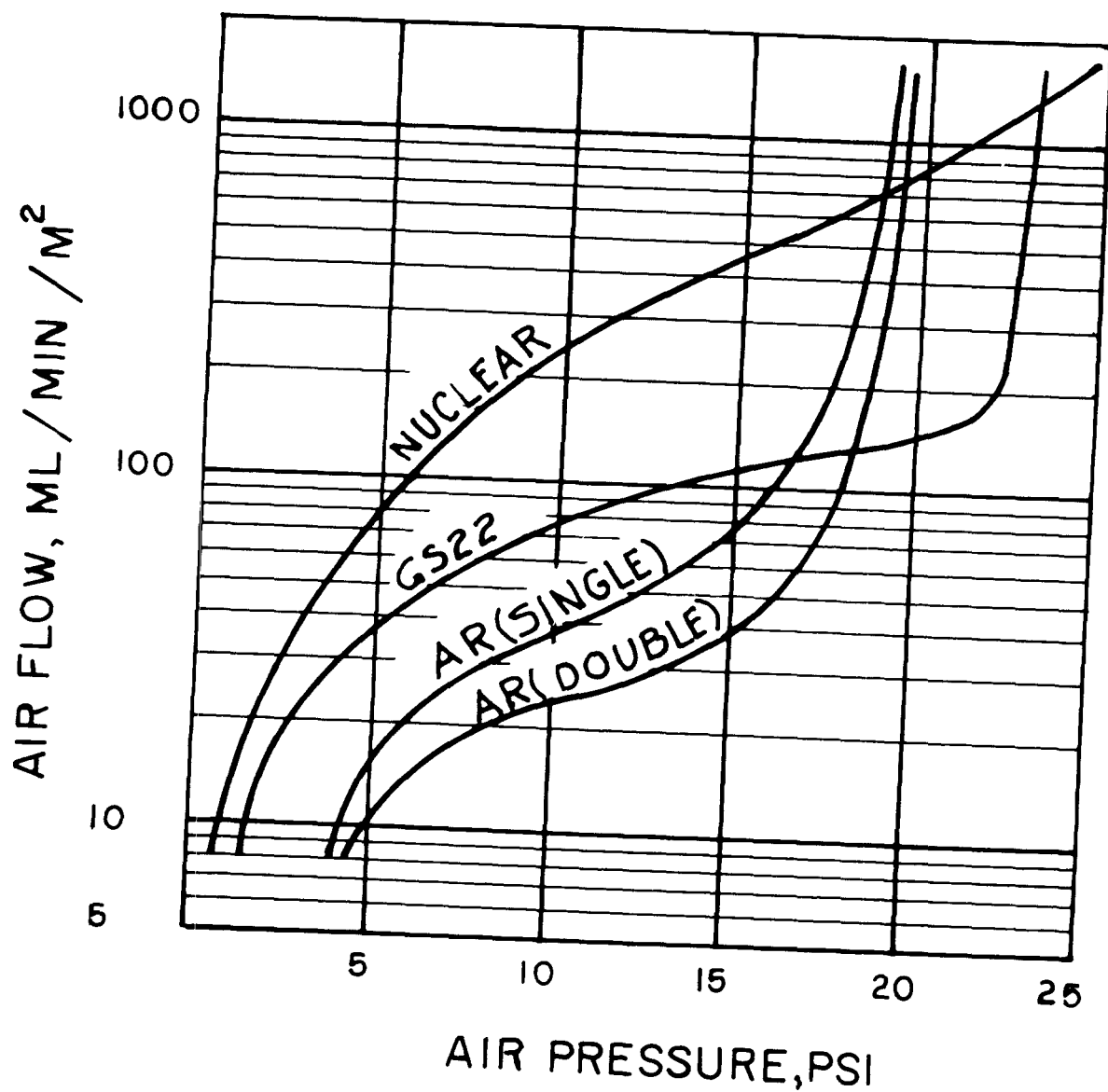


Fig. 1 Flow of air through fibrous, non-fibrous and nuclear type membranes wetted by Varsol.

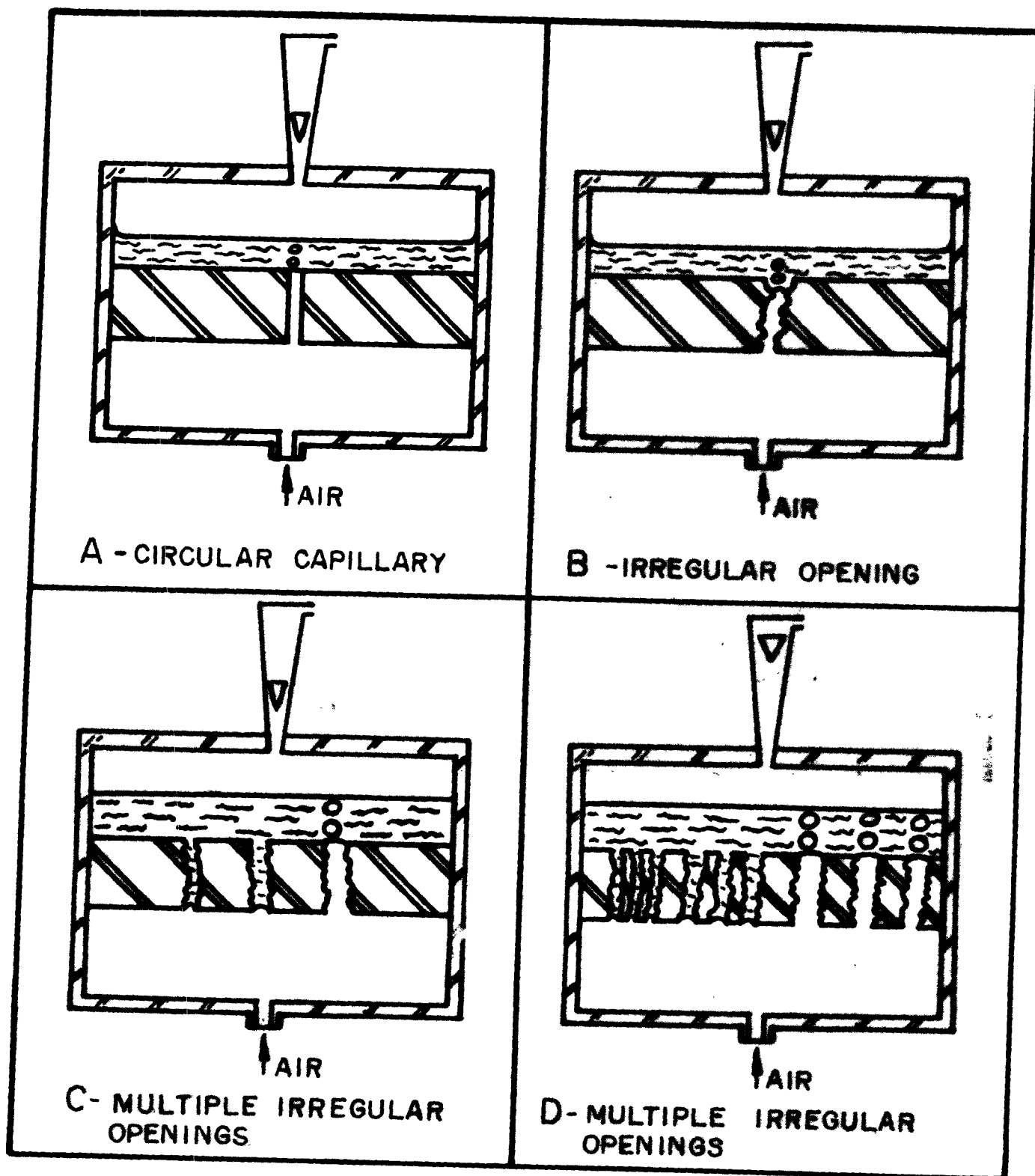
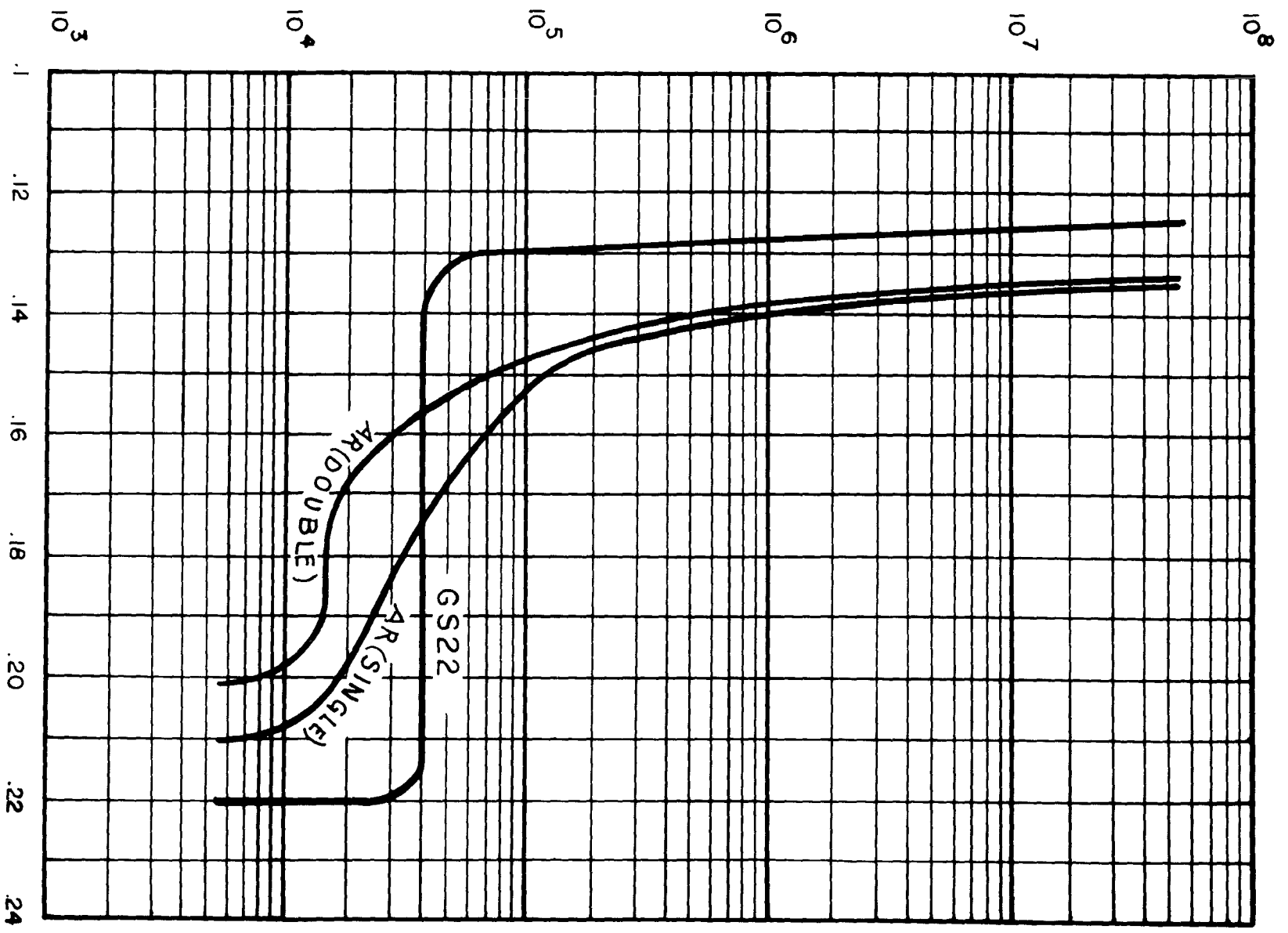


Fig 2. Flow of air through wetted capillaries.

CUMULATIVE NO OF HOLES PER CM² OF FILTER AREA LARGER THAN PORE DIA.



PORE DIA, BY BACTERIOLOGICAL ASSAY, MICROMETERS

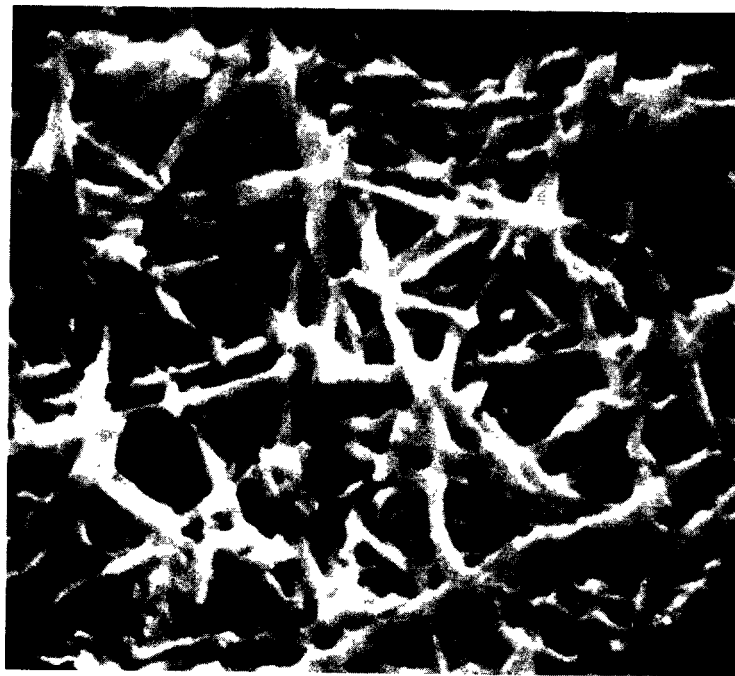


Fig. 4 Scanning electron micrograph of 0.2μ fibrous membrane; 10000X.

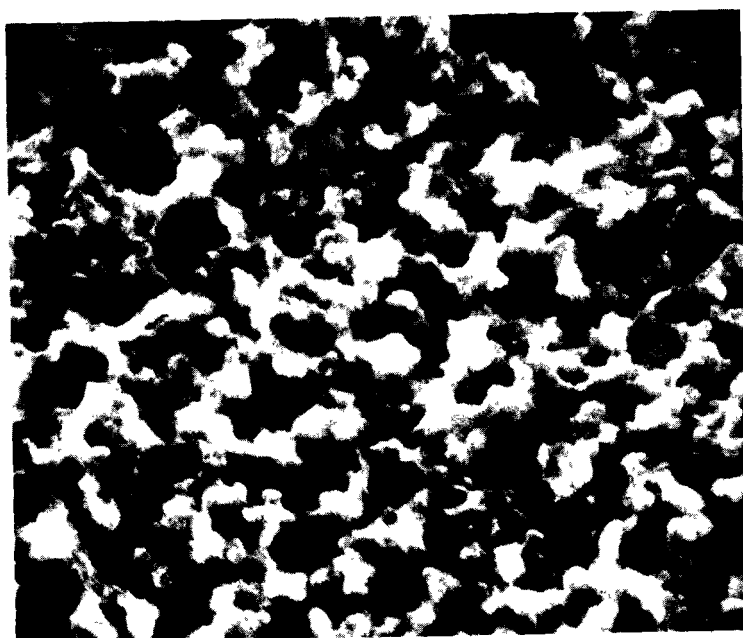
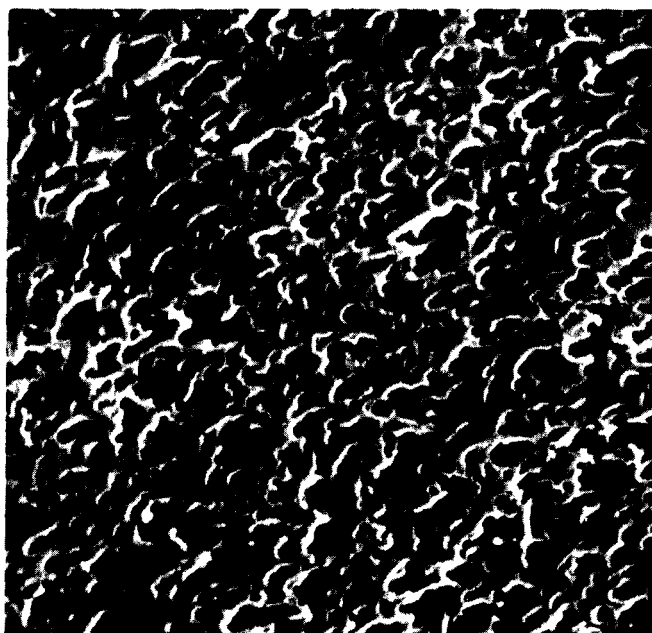
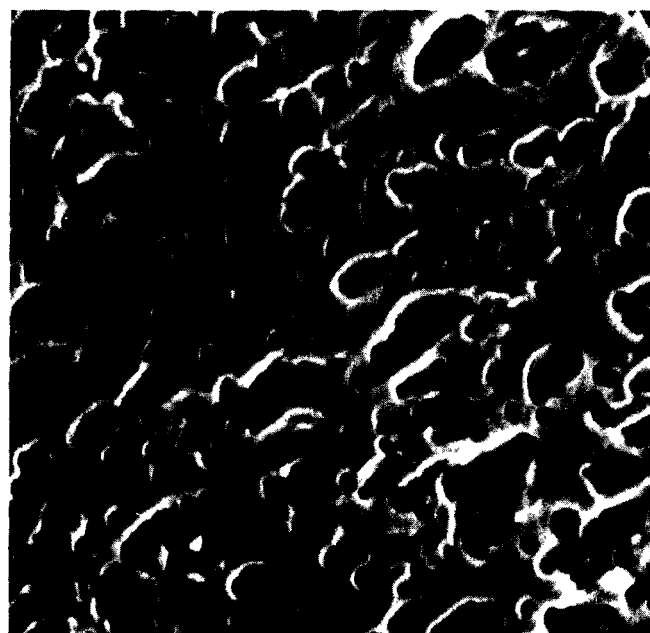


Fig 5 Scanning electron micrograph of GS22-0 22 μ non-fibrous plastic membrane, 10000X.



072573-7A 5,000X.



072573-7B 10,000X.

Fig. 6 Scanning electron micrograph of 0.2 μ nuclear type membrane; 10000X.

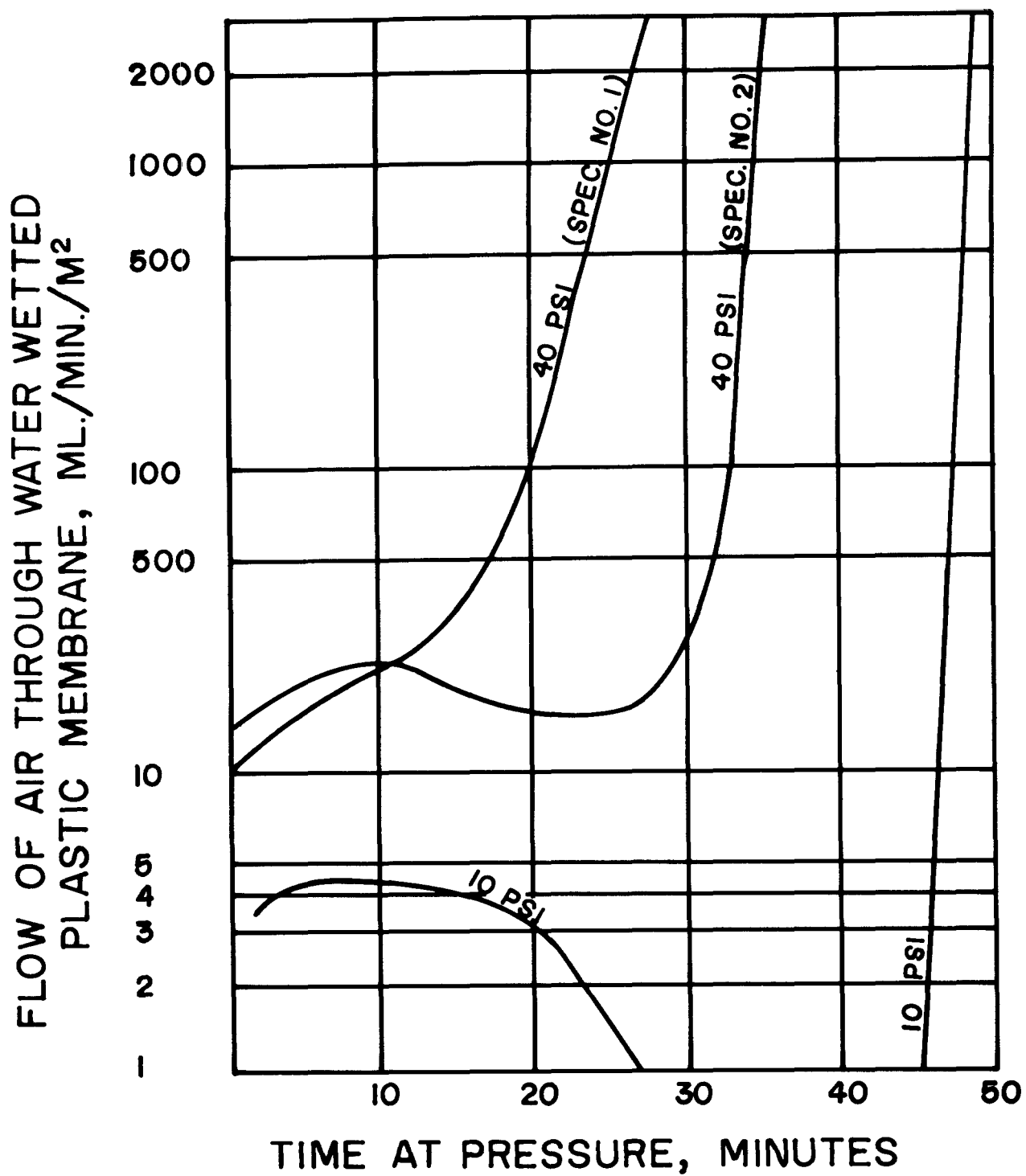


Fig. 7 Flow of air through water wetted GS22 membrane at 10 and 40 psid.

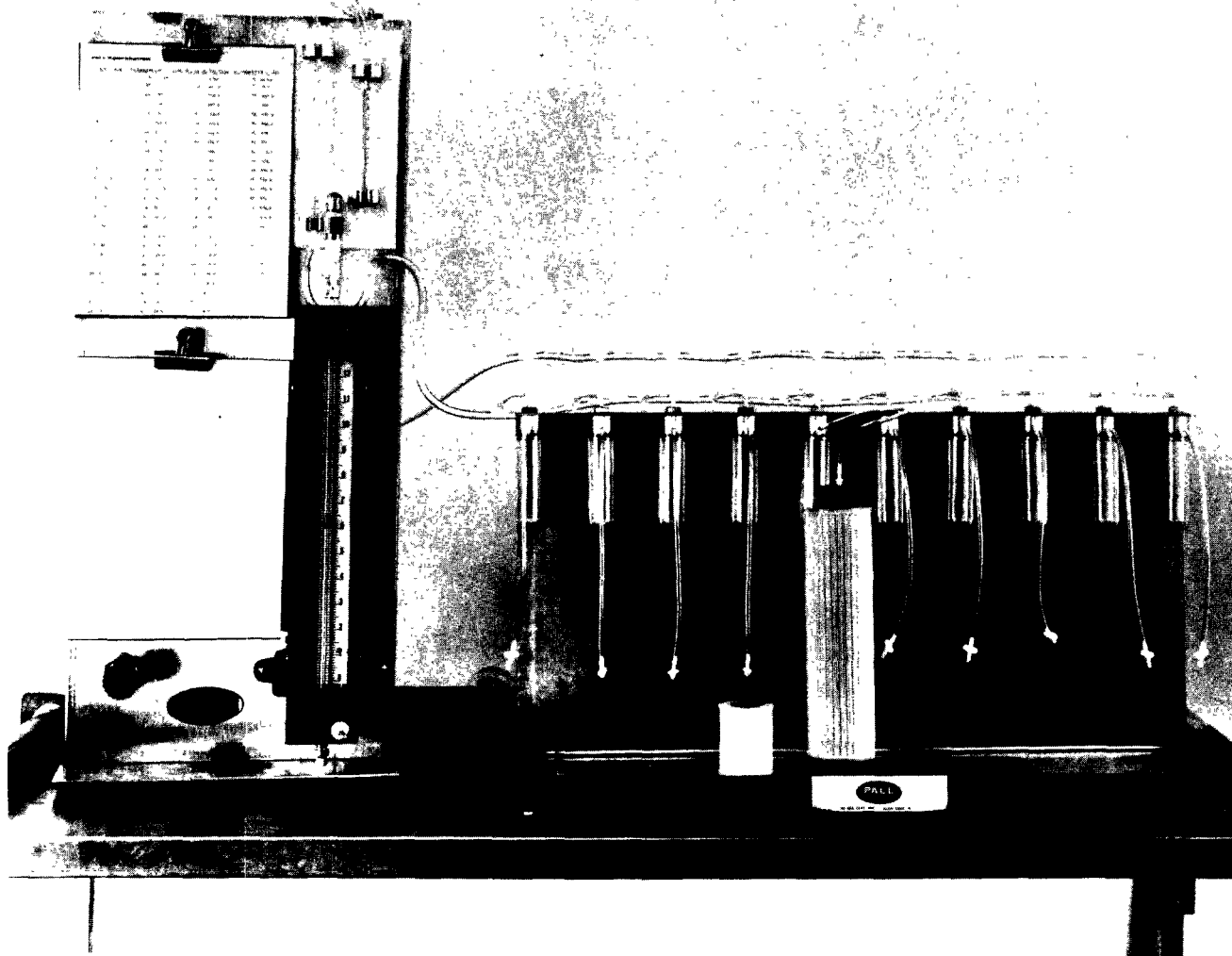


Fig. 8 Apparatus for performing the forward flow bubble point test.

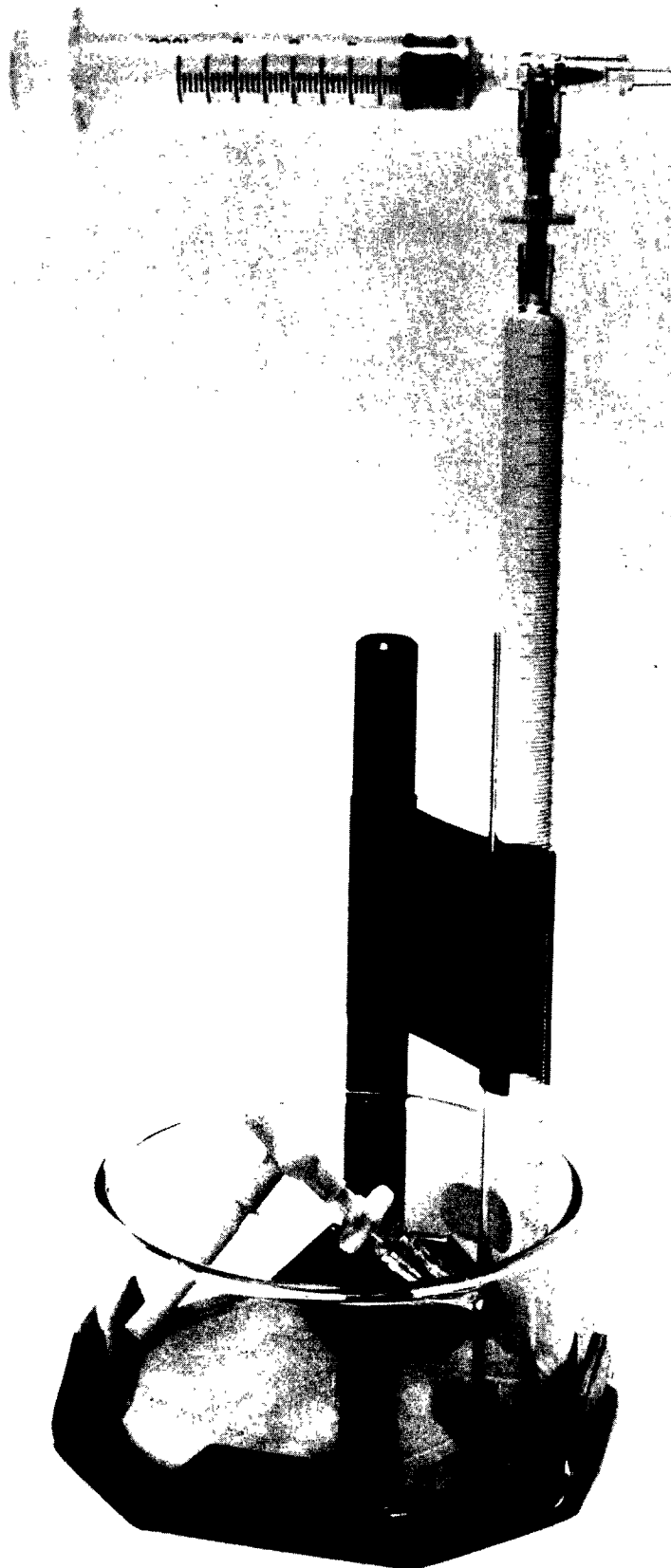


Fig. 9 *Pneumatic trough apparatus for forward flow testing of in-situ sterilized assemblies.*

References:

1. D. B. Pall: U.S. Pat. 3,007,334, 11/7/61
2. D. B. Pall: WADC TR 256, May 1956
3. Millipore Application Manual AM202, p. 35
4. M. Kantor, WIED. ANN. 1892, 4, 399
5. S. Jacobs, Filtration & Separation, Sept. -Oct. 1972, 525
6. Millipore Bulletin MB404, p. 7
7. Handbook of Chemistry & Physics, 42nd Ed., p. 1706-1707
8. E. Kirnbauer, Quality Assurance Practice and Standards for Ultipor^(R) Bacteria Removal Filters, Pall Bulletin AB800-1-72