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STERILIZATION OF LIQUIDS BY FILTRATION AND CERTIFICATION OF PROBABILITY

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ABSTRACT

Four types of hydrosol filters, two reusable (diatomaceous cylinder and fritted glass funnel) and two disposable (asbestos pad and membrane filter), were challenged with a heavy Serratia marcescens suspension to assess their ability to produce sterile filtrates. Two of the four diatomaceous earth filters, the four fritted glass funnels, and all the asbestos pads tested generally gave sterile filtrates. However, only one type of filter, one of the membranes in its manufacturer's own holder, consistently gave sterile filtrates. The two other types of membranes usually gave sterile filtrates if tested in one manufacturer's holder but all types invariably gave contaminated filtrates when tested in another manufacturer's holder. Contaminated filtrates were generally attributed to a poor reusable filter or to a faulty holder used with a disposable filter. If a high degree of certainty is required for sterile heat-labile filtrate, it is suggested that the liquid be passed through two or more filters in a previously tested and proved system.

I. INTRODUCTION

This study was performed under an interagency agreement with the National Aeronautics and Space Administration to determine if filtration of liquid suspensions is a sufficiently dependable sterilization technique to meet the restrictions of current international agreements. These agreements, promulgated under the auspices of the International Committee on Space Research, set acceptable sterilization standards for spacecraft that might intercept Mars or other planets. The standards recognize that sterilization is an absolute term and that one can never be certain of its accomplishment. The goals are therefore expressed in terms of probabilities. The current agreement[1] requires that no spacecraft be launched unless the nation responsible can guarantee that the probability of its placing a viable microorganism on the surface of Mars is less than 1×10^{-4}.

It is currently planned to use dry heat to sterilize the assembled spacecraft. However, certain exobiological experiments now in the planning stage will utilize heat-labile biological fluids that will require sterilization by some other manner before they are added to the spacecraft that has been heat-treated. The most common laboratory method for sterilizing such liquids is filtration. The question then arises whether this technique would require a prohibitive amount of pretesting to demonstrate that the probability of sterility of the biological fluid finally used in the experiment meets the prescribed standards. It is assumed that an investigator would carefully select the type of equipment best adapted to the biological fluid needed for his experiment and that the setup would be pretested to permit calculating the probability of sterility of the final product. This paper attempts to show how such a determination could be made after only a limited amount of pretesting.

Four of the most common types of equipment used for filtration sterilization were given a predetermined number of tests, and the data were then examined for statistical reliability. It should be emphasized that the aim of the experiment was not to determine the reliability of any particular manufacturer's product. It was merely to show, hopefully, that with careful selection of equipment and a limited amount of pretesting filtration could be utilized as a sterilization technique when, because of the sensitivity of the product, this was the preferred method.

Surprisingly, there is little in the literature about the probability that these filters will give a bacteria-free filtrate. Indeed, much of the published work is concerned with differential filtration to separate bacteria from viruses or bacteriophage.[2-4] For a discussion of various precautions, the effect of such factors as the type and pH of the fluid and the porosity and electrical charge or adsorption of the filters, see Sykes[5] or Morton.[6] The literature gives the general impression that failures of filters to give bacteria-free filtrates are by no means uncommon.

The types of hydrosol filters used in these tests were the diatomaceous cylinder, fritted glass funnel, asbestos pad, and the membrane filter. For each test, two 25-ml samples of a heavy bacterial suspension were passed through the filters. One whole filtrate was tested for the presence of microorganisms by adding it to broth to give a qualitative plus or minus result. The second filtrate was tested by plating the entire 25 ml in a large petri dish with nutrient agar for a quantitative colony count. A heavy bacterial suspension was used to challenge each filter in order to obtain significant penetration data.

II. MATERIALS AND METHODS

A. HYDROSOL FILTRATION UNITS

For this comparative study all equipment and supplies were new and were used as they came from the manufacturers. Two of the hydrosol filters, the diatomaceous cylinder and the fritted glass funnel, are designed for reuse, but before each test they must be cleaned and sterilized. The other two, asbestos pads and membrane filters, are discarded after one use, but the holders are reusable. The manufacturers' brief descriptions of the filtration units shown from left to right in Figure 1 are given below:

1) Diatomaceous cylinder (diameter 5/8 inches and length 2½ inches) with a normal or medium porosity connected to a matching glass mantle by washers and a lock nut.

2) Büchner glass funnel with a fritted disc (60-ml capacity) of ultrafine porosity (about 1.2 μ).

3) Asbestos filter (35-mm diameter) with a very fine porosity fitted in a gravity or vacuum silver-plated bronze holder with a center nut (30-ml capacity).

4) Membrane filter (47-mm diameter) with a porosity of about 0.2 μ (obtained from three manufacturers) used in each of two glass holders (300-ml capacity).

Manufacturer A holder: a glass funnel and a base with a coarse fritted glass support held together with an anodized aluminum spring-action clamp.

Manufacturer B holder: a glass funnel and a base with a stainless steel resistance-welded screen support held together with three stainless steel clips attached to the base.

The manufacturers' descriptions of some filters omit specific pore size. Because of irregularities in pore structure, it is impossible to determine true pore size.

Each filtration unit was attached by a rubber stopper to the barrel of a 20-ml syringe fastened to a 20-gauge needle (Fig. 2). The entire assembly was wrapped in paper, sterilized by exposure to ethylene oxide gas for 24 hours, and allowed to stand for a minimum of 2 days before use (to lose any absorbed ethylene oxide). In this way there was no requirement for assembling separate sterile items just before filtration, thus avoiding chances of inadvertent contamination.

B. TEST DESIGN

Table 1 lists the number and types of filters tested, the total number of times each was used, the number of holders tested, and the number of times each holder was used. Each run consisted of testing, in a random order, 12 filtration units; this included two diatomaceous cylinders, two fritted glass funnels, two asbestos pads, and two each of membranes A, B, and C. Thus, 24 runs were required for the total 288 tests conducted. For each of these runs a new bacterial suspension was employed.

C. TEST PROCEDURE

The bacterial suspension used for each run was prepared by adding Serratia marcescens cells washed from a 24-hour agar culture slant to about 750 ml of tap water previously boiled to remove the chlorine. The viable count did not change appreciably when the test organism was suspended in boiled tap water for the 3-hour test period, but there was a 5-log decrease in count in distilled water. Each filtration unit was mounted in an upright position (Fig. 2). Two 25-ml samples of bacterial suspension, each containing about 10 million cells, were filtered into separate sterile rubber-stoppered bottles by reducing the pressure to approximately 10 cm mercury through a 20-gauge needle inserted into each bottle before it was sterilized. One bottle containing 25 ml of double-strength trypticase soy broth without dextrose was used to test the filtrate qualitatively for bacterial growth. The other bottle was empty and was used to collect 25 ml of filtrate for quantitative viable count. A random order was used to determine which sample would be cultured in broth and which would be assayed in agar.

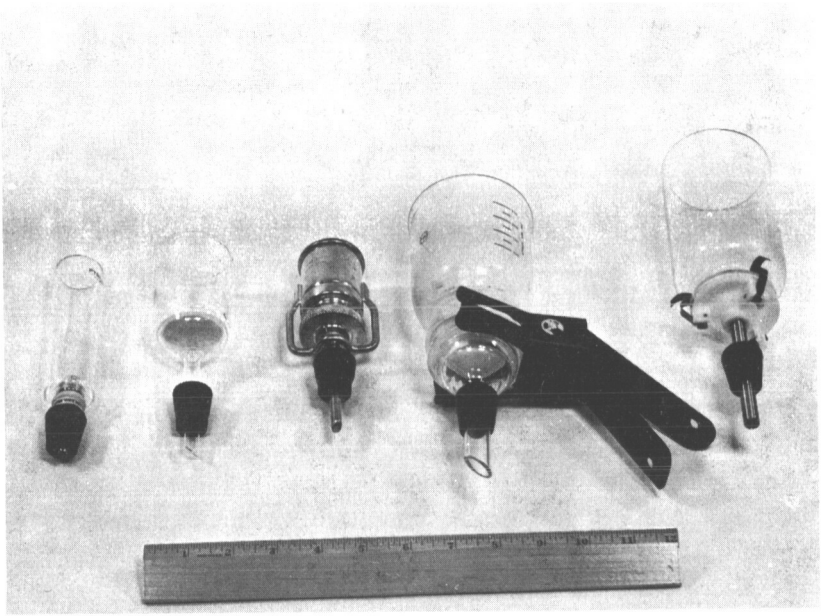

Figure 1. Filtration Units Tested. See text for descriptions.

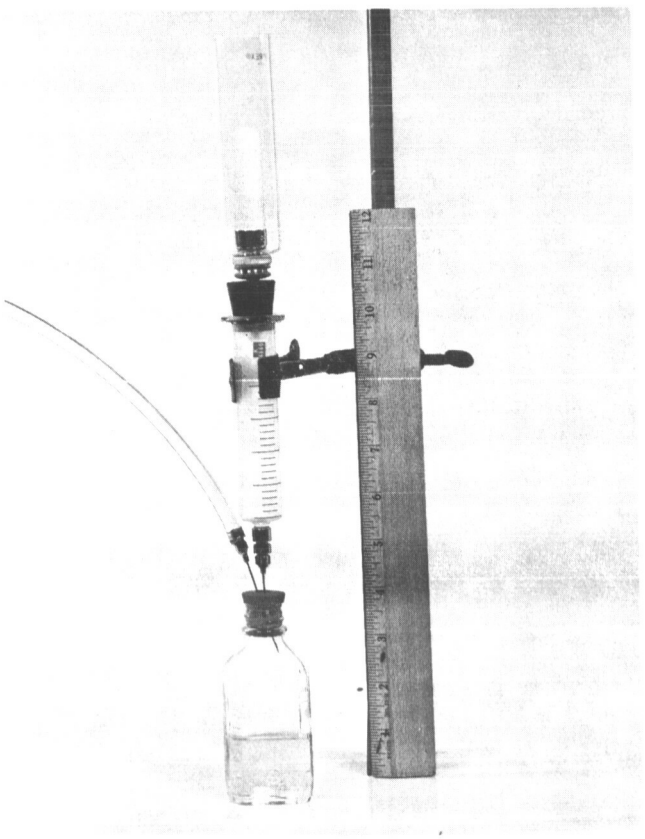

Figure 2. Filtration Unit Mounted.

TABLE 1. SCHEMA OF HYDROSOL FILTRATION TESTS

Filtration Unit		Number of Filters		Number of Holders		Total Number of Tests
Filter	Holder	Total	Times Used	Total	Times Used	
1) Diatomaceous cylinder	Glass mantle[a]	4	12	4	12	48
2) Fritted glass	Büchner funnel[a]	4	12	4	12	48
3) Asbestos pad	Bronze	48	1	4	12	48
4) Membrane A	Glass A[b]	24	1	4	6	24
	Glass B[c]	24	1	4	6	24
Membrane B	Glass A[b]	24	1	4	6	24
	Glass B[c]	24	1	4	6	24
Membrane C	Glass A[b]	24	1	4	6	24
	Glass B[c]	24	1	4	6	24

a. Holder always tested with the same filter.
b. The same four holders from Manufacturer A were used to test all membranes.
c. The same four holders from Manufacturer B were used to test all membranes.

For the quantitative assay, the entire 25 ml of filtrate was removed from the bottle with a needle and syringe and placed in a large petri dish. A pour plate was prepared with trypticase soy agar. All plates were incubated at 32 C for 24 to 72 hours before counting colonies. The number of colonies per plate and the degree of coalescence determined the incubation period. The filtrate in the broth was incubated at 32 C for 7 days before checking for bacterial growth. Control counts were also done on each bacterial suspension both before the filtrations began and again after their completion.

III. RESULTS AND DISCUSSION

The results of this study are shown in Tables 2 through 7. Raw data are presented rather than means so that the peculiarities that occurred can be clearly shown. It is readily apparent that all four types of hydrosol filters could and did produce sterile filtrates. However, contaminated filtrates were also obtained with each type of filter unit with the exception of membrane A used in the four holders from Manufacturer A (Table 5).

The efficiency of the four diatomaceous cylinders tested varied greatly (Table 2). Most of the filtrates were contaminated when filter 1 or 4 was used, but with filters 2 or 3, sterile filtrates were obtained in all but two instances. Here is clear evidence that it can not be assumed that equipment as received will perform satisfactorily.

The results with fritted glass (Table 3) and the asbestos pads (Table 4) were generally excellent. Bacterial growth occurred only seven times. Four times, however, the organism was not S. marcescens. This occurred twice in the first four filtrations with asbestos pad holder 1. Because of the exceptionally long filtration time, this holder was examined and found to have a minute hole that allowed air to enter behind the asbestos pad itself. The filtrate thus could become recontaminated with an airborne organism or organisms after filtration. No such contamination occurred after the hole was repaired between the fourth and fifth filtration. No obvious flaw was evident with the two fritted glass funnels where this same phenomenon occurred, nor on the one occasion when this showed up with one of the membrane filter combinations (Table 6). Extreme care was taken when designing and conducting these tests to use aseptic techniques so that outside contamination would not occur. Even so, in these few instances an unexplained contaminant appeared. It is suspected that these contaminations, too, occurred after filtration, possibly because of faulty equipment, an air leak in the system, or even a break in the aseptic technique.

TABLE 2. NUMBER OF S. MARCESCENS ORGANISMS PENETRATING DIATOMACEOUS FILTERS

Test No.	Filter 1			Filter 2			Filter 3			Filter 4		
	Org. per 25 ml Filtrate	25 ml Filtrate in Broth[a]	Time,[b] sec.	Org. per 25 ml Filtrate	25 ml Filtrate in Broth[a]	Time,[b] sec.	Org. per 25 ml Filtrate	25 ml Filtrate in Broth[a]	Time,[b] sec.	Org. per 25 ml Filtrate	25 ml Filtrate in Broth[a]	Time,[b] sec.
1	3	+	86	0	-	136	1	-	72	17	-	90
2	>10,000	+	85	0	-	52	0	-	57	6	-	90
3	230	+	94	0	-	56	0	-	60	16	+	97
4	2,300	+	94	0	-	60	0	-	56	0	-	94
5	2,900	+	95	0	-	59	0	-	62	920	+	96
6	920	+	102	0	-	58	2	-	72	1	+	93
7	130	+	123	0	-	57	0	-	66	>10,000	-	102
8	300	+	130	0	-	60	0	-	63	0	+	108
9	[c]	+	77	0	-	62	0	-	68	15	+	105
10	2,500	+	142	0	-	57	0	-	72	0	+	113
11	100	+	144	0	-	59	0	-	72	0	+	108
12	72	+	172	0	-	64	0	-	75	16	+	113

a. +, S. marcescens present; -, no bacterial growth.
b. Average time required to filter 25 ml of bacterial suspension (about 10 million cells) at 10 cm mercury pressure.
c. Sample lost.

TABLE 3. NUMBER OF S. MARCESCENS ORGANISMS PENETRATING FRITTED GLASS FUNNELS

Test No.	Funnel 1			Funnel 2			Funnel 3			Funnel 4		
	Org. per 25 ml Filtrate	25 ml Filtrate in Broth[a]	Time,[b] sec.	Org. per 25 ml Filtrate	25 ml Filtrate in Broth[a]	Time,[b] sec.	Org. per 25 ml Filtrate	25 ml Filtrate in Broth[a]	Time,[b] sec.	Org. per 25 ml Filtrate	25 ml Filtrate in Broth[a]	Time,[b] sec.
1	0	-	390	0	-	192	0	-	282	0	-	248
2	0	-	260	0	-	196	0	-	211	0	-	254
3	0	-	256	0	-	207	0	-	225	0	-	254
4	0	±	280	0	-	200	0	-	270	0	±	272
5	0	-	288	0	-	203	0	-	224	0	-	255
6	0	-	298	0	-	204	0	-	241	0	-	267
7	0	-	256	0	-	212	0	-	253	0	-	273
8	0	-	272	0	-	208	0	-	237	0	-	260
9	0	-	260	0	-	208	0	-	231	0	+	271
10	0	-	270	0	-	220	0	-	225	0	-	262
11	0	-	238	0	-	222	0	-	230	0	-	256
12	0	-	244	0	-	204	0	-	234	0	-	224

a. ±, bacterial growth but not S. marcescens; +, S. marcescens present; -, no bacterial growth.
b. Average time required to filter 25 ml of bacterial suspension (about 10 million cells) at 10 cm mercury pressure.

TABLE 4. NUMBER OF S. MARCESCENS ORGANISMS PENETRATING ASBESTOS PADS IN FOUR BRONZE HOLDERS

Test No.	Holder 1			Holder 2			Holder 3			Holder 4		
	Org. per 25 ml Filtrate	25 ml Filtrate in Broth[a]	Time,[b] sec.	Org. per 25 ml Filtrate	25 ml Filtrate in Broth[a]	Time,[b] sec.	Org. per 25 ml Filtrate	25 ml Filtrate in Broth[a]	Time,[b] sec.	Org. per 25 ml Filtrate	25 ml Filtrate in Broth[a]	Time,[b] sec.
1	0	±	1012[c]	0	-	113	0	-	140	0	-	165
2	0	-	1029[c]	0	-	131	0	-	144	0	-	142
3	0	±	1255[c]	0	-	193	0	-	230	0	-	159
4	0	-	980[c]	0	-	149	0	-	160	0	-	164
5	0	-	203	0	-	159	0	-	203	0	-	174
6	0	-	152	0	-	180	0	-	194	1	-	160
7	0	-	170	0	-	152	0	-	186	0	-	185
8	0	-	154	0	-	134	0	-	166	0	-	143
9	0	-	150	0	-	141	0	-	187	0	-	166
10	1	-	167	0	-	162	0	-	179	0	-	172
11	0	-	192	0	-	166	0	-	152	0	-	148
12	0	-	182	0	-	159	0	-	190	0	-	134

a. ±, bacterial growth but not S. marcescens; -, no bacterial growth.
b. Average time required to filter 25 ml of bacterial suspension (about 10 million cells) at 10 cm mercury pressure.
c. A minute hole was discovered and repaired after test 4.

With the membrane filter, the holder appeared to be at least as critical as the filters (Tables 5, 6 and 7). All three types of membrane filters performed well in the four holders obtained from Manufacturer A; all three performed poorly at first in the four holders from Manufacturer B, but the performance improved when three binder clips were used in addition to the clips attached to the base of the holder. In the latter holder, membrane A appeared to be better than membrane B, which in turn was better than membrane C. The same distinction among membranes from different manufacturers is harder to make in the better holders. Manufacturer B's membranes in Manufacturer A's holders performed almost as well as Manufacturer A's membranes in Manufacturer A's holders, and were less efficient only in their own holders.

Most of the time the results obtained for each two 25-ml samples of filtrate were comparable whether assayed by pour plate method or cultured in broth. They were both negative in 197 tests, both positive in 69. This is to be expected because a whole 25-ml sample was used for the pour plate as well as for the broth culture, and the sensitivity of the two methods should be comparable. In the six instances in which there were contaminated broth samples but sterile pour plates, growth in the broth presumably was caused by the presence of only a few microorganisms, none of which showed up by chance alone in the pour plate. This explanation is also valid for the times when only a few S. marcescens colonies were detected in the pour plate and the comparable broth sample was sterile. The five times that sterile broth samples were obtained but significant counts of 17, 90, or more than 10,000 S. marcescens colonies appeared on the pour plate are much more difficult to explain. In each of these five puzzling instances, however, the second filtrate, by chance, was the one used for the agar plates. The chance of microorganisms slowly working their way through a filter, as more and more contaminated fluid is filtered, was mentioned briefly by both Morton[6] and Sykes[5] and may be the answer here. In reality, 50 ml is probably considerably more than will be used in a spacecraft experiment.

TABLE 5. NUMBER OF S. MARCESCENS ORGANISMS PENETRATING MEMBRANE A IN TWO TYPES OF HOLDERS

Holder No.	Test No.	Mfg. A Holder			Mfg. B Holder[a]		
		Org. per 25 ml Filtrate	25 ml Filtrate in Broth[b]	Time,[c] sec.	Org. per 25 ml Filtrate	25 ml Filtrate in Broth[b]	Time,[c] sec.
1	1	0	-	25	11	-	24
	2	0	-	25	>10,000	+	22
	3	0	-	28	0	+	22
	4	[d]	[d]	26	0	-	21
	5	0	-	26	0	-	20
	6	0	-	24	0	-	22
2	1	0	-	26	1	+	24
	2	0	-	30	240	+	21
	3	0	-	27	96	+	20
	4	0	-	27	0	-	20
	5	0	-	28	0	-	21
	6	0	-	30	60	+	21
3	1	0	-	21	0	-	22
	2	0	-	23	710	+	24
	3	0	-	22	22	+	23
	4	0	-	22	>10,000	+	28
	5	0	-	22	0	-	22
	6	0	-	26	0	-	24
4	1	0	-	46	1	+	20
	2	0	-	27	90	-	22
	3	0	-	27	0	+	22
	4	0	-	24	0	-	25
	5	0	-	28	0	-	24
	6	0	-	25	0	-	22

a. For Mfg. B holders in tests 4, 5, and 6, three binder clips were used in addition to the clips attached to the base of the holder.
b. +, S. marcescens present; -, no bacterial growth.
c. Average time required to filter 25 ml of bacterial suspension (about 10 million cells) at 10 cm mercury pressure.
d. Sample lost.

TABLE 6. NUMBER OF S. MARCESCENS ORGANISMS PENETRATING MEMBRANE B IN TWO TYPES OF HOLDERS

Holder No.	Test No.	Mfg. A Holder			Mfg. B Holder[a]		
		Org. per 25 ml Filtrate	25 ml Filtrate in Broth[b]	Time,[c] sec.	Org. per 25 ml Filtrate	25 ml Filtrate in Broth[b]	Time,[c] sec.
1	1	0	-	44	2,200	+	37
	2	0	-	32	2,300	+	31
	3	0	-	38	260	+	36
	4	0	-	36	600	+	32
	5	0	-	25	0	-	27
	6	0	-	30	0	-	38
2	1	0	-	44	1,500	+	44
	2	0	±	38	460	+	32
	3	0	-	60	2,300	+	35
	4	0	-	40	>10,000	+	64
	5	0	-	30	1,200	+	28
	6	0	-	28	0	-	24
3	1	3	+	33	3,500	+	42
	2	0	-	30	>10,000	+	42
	3	0	-	50	920	+	42
	4	0	-	27	0	-	42
	5	0	-	22	13	+	26
	6	0	-	38	1	+	28
4	1	0	-	36	390	+	44
	2	0	-	36	210	+	42
	3	0	-	56	53	+	44
	4	0	-	31	0	-	44
	5	0	-	27	0	-	28
	6	0	-	25	400	+	40

a. For Mfg. B holders in tests 4, 5, and 6, three binder clips were used in addition to the clips attached to the base of the holder.

b. -, no bacterial growth; +, S. marcescens present; ±, bacterial growth but not S. marcescens.

c. Average time required to filter 25 ml of bacterial suspension (about 10 million cells) at 10 cm mercury pressure.

TABLE 7. NUMBER OF S. MARCESCENS ORGANISMS PENETRATING MEMBRANE C IN TWO TYPES OF HOLDERS

Holder No.	Test No.	Mfg. A Holder			Mfg. B Holder[a]		
		Org. per 25 ml Filtrate	25 ml Filtrate in Broth[b]	Time,[c] sec.	Org. per 25 ml Filtrate	25 ml Filtrate in Broth[b]	Time,[c] sec.
1	1	3	+	46	>10,000	+	42
	2	5	+	48	>10,000	+	40
	3	0	-	48	>10,000	+	42
	4	0	-	48	0	-	42
	5	0	-	50	>10,000	+	44
	6	0	-	52	>10,000	+	44
2	1	0	-	57	>10,000	+	41
	2	0	-	52	>10,000	+	42
	3	0	-	50	>10,000	+	38
	4	0	-	52	0	-	44
	5	0	-	52	>10,000	+	40
	6	0	-	48	>10,000	+	38
3	1	>10,000	+	44	>10,000	+	42
	2	0	-	44	>10,000	+	47
	3	0	-	44	>10,000	+	46
	4	0	-	44	>10,000	+	45
	5	0	-	47	>10,000	+	45
	6	0	-	43	>10,000	+	54
4	1	0	-	54	>10,000	+	48
	2	0	-	101	>10,000	+	43
	3	0	-	56	>10,000	+	42
	4	0	-	44	>10,000	+	43
	5	0	-	46	17	+	42
	6	0	-	52	500	+	44

a. For Mfg. B holders in tests 4, 5, and 6, three binder clips were used in addition to the clips attached to the base of the holder.
b. -, no bacterial growth; +, S. marcescens present.
c. Average time required to filter 25 ml of bacterial suspension (about 10 million cells) at 10 cm mercury pressure.

Another general observation is that contamination, when it occurred, was generally low except in a few instances where more than 10,000 colonies were found; most of these were with membrane C used in Manufacturer B's holder (Table 7). Although the numbers may appear high, the challenge was approximately 1×10^7 organisms. The measurable leakages were all under 0.01% and most were far lower. The data also indicated that sterile filtrates could consistently be obtained with all four types of filters in certain instances. It should be remembered that in these tests the filters were challenged with very large numbers of microorganisms; the fluids actually used in space tests will probably contain only a few if any microorganisms before filtration.

There is no clear-cut evidence from the data that any one of these filtration techniques is much more efficient than the others. Each system gave some sterile filtrates and, with each, there was failure to sterilize. Thus, when choosing the filter, holder, and auxiliary apparatus, one can take into consideration other factors such as the rate of filtration and the characteristics of the biological fluid to be filtered. Some types of filters shown to be satisfactory for an aqueous bacterial suspension may be rejected because of technical difficulties occurring when a specific biological fluid is filtered.

After selecting a technique, the specific items of equipment must be pretested under conditions more stringent than those that will occur in the actual experiment in order to give assurance that the system is satisfactory. The data accumulated on pretested apparatus will then provide the basis of determining whether the biological fluid filtered for any critical experiment would meet the established statistical requirements for sterility.

As an example, the raw data given here were examined to determine what expression of confidence could be given for a final sterile filtrate. The analysis shows that the problem requires two separate treatments: one for variation in the same filter used numerous times (the diatomaceous and fritted glass filters), the other for the variation of the same filter holder used numerous times but each time with a different disposable filter but one from the same manufacturer's lot (the asbestos and membrane filters). In the latter instance the question of variation within lots enters, which could result in a very large number of trials; however, experience indicates that the probability of drawing a poor disposable filter from a good lot is small.

For the better reusable filters, such as the diatomaceous filter 2 or fritted glass funnels 2 and 3, sterile filtrates were obtained in all 12 trials with a test dose of 20,000,000 cells per trial or 240,000,000 in toto. Assuming a large series of trials with 240,000,000 organisms each, with the logical supposition of a Poisson distribution of organisms passing, it may be shown that a mean (M) of three will occasionally yield a sample result of zero. Means of four or more will very rarely give zero. This may be shown either by solving the Poisson expression e^{-m} $(1, M, M^2/2!, M^3/3!,$ etc.)

as described by Fisher[7] or by using published tables. It may thus be inferred that the true mean passing for 240,000,000 cells was not over three; this may be taken as a tentative maximum. Some of the other good filters with very small numbers passing fall close to this estimate. With 80 organisms in the material to be filtered and a maximum of one chance in 80,000,000 organisms passing, this indicates one chance in 1,000,000 of any passing. For the selected reusable filters, a reasonable statistical assurance of sterility can be given based only upon the assumption that microorganisms are filtered out in the same ratio in low- as in high-titer suspensions, and that the filter was not mechanically or otherwise damaged between its last test and the time it was put to actual use.

The same type of calculation applies for the pretested holder with a disposable filter from a lot shown to contain good filters, but an additional assumption must be made that the lot will not contain any defective filters. Considerably more testing will be required to provide assurance that a disposable filter selected from a lot previously shown to be highly efficient will itself be as efficient as the others.

Confidence can be increased if two or more filters are used in succession. If, for example, there is one chance in 1,000 of getting a defective filter out of any lot, there will be one chance in a million of getting two defective ones in succession, and one chance in a billion of three defective ones in succession.

Because double filtration presents no great experimental problem, it can be recommended that for critical exobiological experiments, where it is highly important that the liquid biological detection medium be sterile, a tested and proved system be preselected utilizing two or more filtrations. In such an experimental setup, a sufficiently high probability of sterility should be apparent to meet the restraints of this international agreement, without requiring a prohibitive number of pretests.

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