RETTGER, L. F. 1910 A new and improved method of enumerating air bacteria. J. Med. Research, **22**, 461–468.
ROSEBURY, T. 1947 Experimental air-borne infection. In *Microbiological Monographs*. The Williams & Wilkins Co., Baltimore, Maryland.
SHIPE, E. L., TYLER, M. E., AND CHAPMAN, D. M. 1959 Bacterial aerosol samplers. II. Development and evaluation of the Shipe sampler. Appl. Microbiol., **7**, 349–354.
SONKIN, L. S. 1951 Role of particle size in experimental airborne infection. Am. J. Hyg., **53**, 337–354.
SPENDLOVE, J. C. 1957 Production of bacterial aerosols in a rendering plant process. Public Health Repts. (U. S.), **72**, 176–180.
TYLER, M. E., SHIPE, E. L., AND PAINTER, R. B. 1959 Bacterial aerosol samplers. III. Comparison of biological and physical effects in liquid impinger samplers. Appl. Microbiol., **7**, 355–362.
WELLS, W. F. 1933 Apparatus for study of the bacterial behavior of air. Am. J. Public Health, **23**, 58–59.
WELLS, W. F. 1955 *Airborne contagion and air hygiene*. Harvard University Press, Cambridge, Massachusetts.
WELLS, W. F., WINSLOW, C. E. A., AND ROBERTSON, E. C. 1946 Bacteriological procedures in the evaluation of methods for control of airborne infection. Am. J. Public Health, **36**, 324.
WHEELER, S. M., FOLEY, G. E., AND JONES, J. D. 1941 A bubbler pump method for quantitative estimations of bacteria in the air. Science, **94**, 445–446.

Bacterial Aerosol Samplers

II. Development and Evaluation of the Shipe Sampler

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Several criteria for a good biological aerosol sampler were suggested by Tyler and Shipe (1959). Certain of these characteristics were taken into consideration in the development of a sampler which might avoid certain deficiencies in existing devices; the Shipe sampler represented the culmination. At the inception of the work, the opinion of the authors was that direct impingement of vegetative bacteria against the bottom of the all-glass impinger at near sonic velocity might result in killing or injuring cells to the extent that the sample would not be an index of the viable cells entering the sampler. A second consideration was that some particles present in a heterogeneous aerosol (>3.0 μ MMD[3]) were of such a size as to be retained by the intake tube on the usual impinger type sampler. The relative infectivity of bacterial particles of various sizes is debatable; our purpose was to determine whether discrimination occurred in various samplers. We felt that it was important to know what was happening to the total aerosol, that is, whether the cells are retained, killed, or sampled.

Presented here are data comparing the Shipe sampler with other types of impinger samplers (all-glass impinger, capillary impinger, Midget impinger, Venturi scrubber, and standard impinger) as described by Tyler and Shipe (1959) under a variety of conditions involving the sampling of aerosols of heterogeneous (>3.0 μ MMD) and homogeneous (<3.0 μ MMD) particle sizes. In nearly all instances with vegetative bacteria, the Shipe sampler collected more viable cells per L of aerosol sampled, and exhibited a low slippage excelled only by the all-glass impinger. This may have been due to one or both of the following: absence of particulate retention in the absence of an intake tube, or minimizing of cellular damage due to impingement. Data supporting these hypotheses are presented in the third paper of this series Tyler *et al.* (1959).

MATERIALS AND METHODS

Design of Shipe sampler. The initial design of the Shipe sampler is shown in figure 1. It should be noted that, by using a metal disk with a bored, sharp-edged orifice, the intake tube was eliminated. Theoretically, any particle smaller than the orifice could enter the sampler. The neck of the orifice support tube entered a 125-ml Erlenmeyer flask perpendicular to the circumference and tangential to the bottom of the flask. The sampling rate was controlled (a) by the size of the orifice and (b) by maintaining a pressure differential greater than one-half an atmosphere across the orifice by means of an adequate vacuum source connected to the exhaust tube of the sampler. The sampler contained 25 ml of gelatin-phosphate diluent plus approximately 0.02 ml of sterile olive oil to minimize foaming. The action of the liquid in such a sampler was quite violent, and this design was abandoned in favor of the one shown in figure 2. Here the orifice support tube entered the flask tangentially to both the circumference and the bottom

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[3] MMD = mass median diameter (May, 1945).

of the flask. The action here was less violent, resulting in a circular motion of the sampling fluid within the flask and a cyclonic action within the orifice support tube. Essentially, all of the trials described were performed with this sampler. A further refinement resulted in the adapting of the orifice tube to what was essentially the base of the all-glass impinger. Such a sampler is shown in figure 3. Splashing was minimized, and comparisons of the latter two samplers indicated that they yielded similar results.

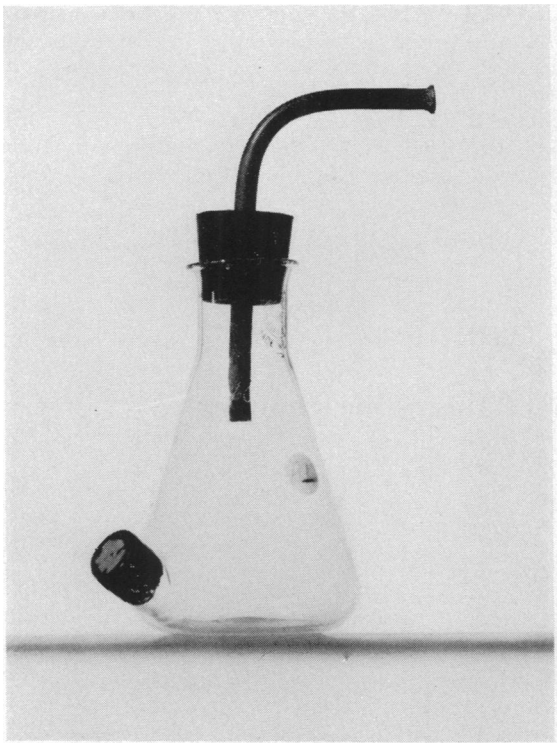

Figure 1. Shipe sampler in initial design. Note support tube perpendicular to circumference of flask.

One of the difficulties encountered in design and construction of the Shipe sampler was the attachment of the metal orifice to the glass intake tube. Several adhesives were investigated but none was found entirely satisfactory; difficulty was encountered in the sterilization of the unit. However, since the time of the work described, a unit has been fabricated out of nylon which overcomes this difficulty.

Evaluation techniques. The bacterial suspensions, aerosol chambers, aerosol samplers, disseminators, bacterial assay techniques, and statistical analyses were described previously (Tyler and Shipe, 1959). One series of studies involved the use of *Escherichia coli*; suspensions were prepared in a manner essentially similar to that for *Serratia marcescens*. In addition, several trials were performed in which *S. marcescens* was disseminated with an explosive device from the center point of a circular, outdoor grid area on which sampling stations were spaced in arcs 50 and 100 yards from the point of dissemination. Parallel samples were taken at each of the sampling stations and assayed for cell count by bacteriological plating techniques.

RESULTS

Removal efficiency. The effectiveness of separation of bacteria from air was tested with the Shipe sampler in

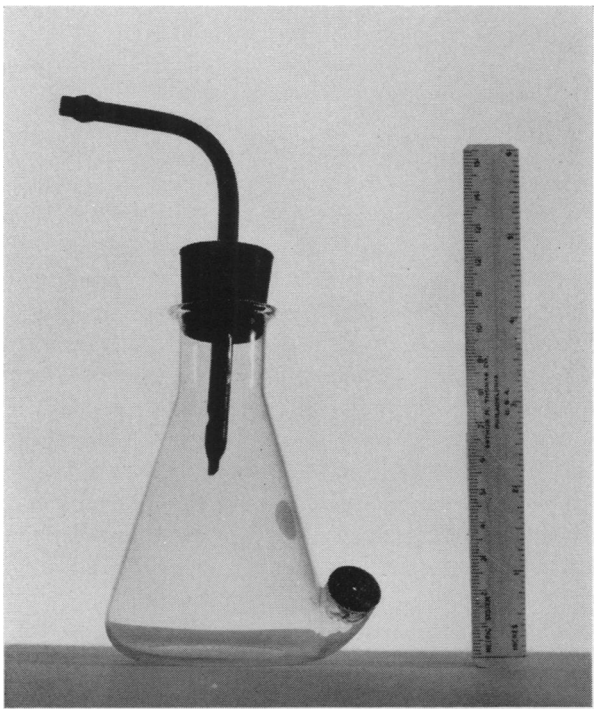

Figure 2. Shipe sampler. Note orifice tangential both to bottom and circumference of flask. Operated near sonic velocity at 8.5 L air per min; 25 ml fluid.

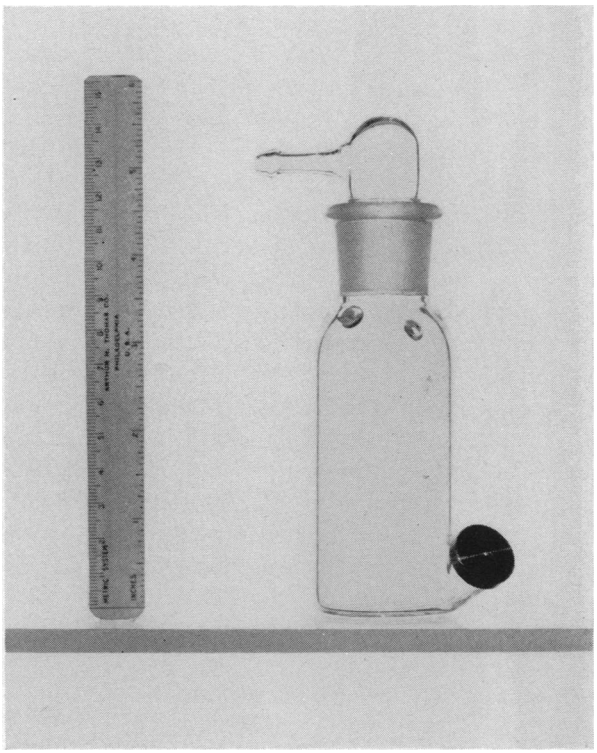

Figure 3. Modified Shipe sampler

a manner analogous to that reported for other samplers (Tyler and Shipe, 1959). Samples of an aerosol of *Bacillus subtilis* spores (<3.0 μ MMD) were taken with a Shipe sampler containing 25 ml of fluid. A cotton sampler was attached in tandem to the exhaust of the sampler. Spores collected in the cotton sampler were assumed to represent those that were not entrapped in the Shipe sampler. A typical trial is illustrated in table 1. The average loss from the exhaust in 64 trials was 3.8 ± 0.2 per cent of the total collected by sampling fluid plus cotton. This efficiency compared favorably with that of most other bacterial samplers reported in the literature except that of the all-glass impinger (Tyler and Shipe, 1959).

Comparison of samplers for collection of aerosols <3.0 μ MMD. Eighteen comparisons were made between the Shipe sampler and the all-glass impinger for the collection of *S. marcescens* aerosol disseminated in the glass tube aerosol unit previously described (Tyler and Shipe, 1959). The DeVilbiss[4] no. 40 nebulizer was used to disseminate the aerosol. Sampling time was of 1-min duration at approximately 1-min intervals. Six sets of samples were taken from one continuous dissemination. Table 2 illustrates the type of comparative results obtained. In 18 comparisons, the Shipe sampler gave a plate count of 1,400,000 ± 60,000[5] per L of aerosol as compared to 1,000,000 ± 35,500 per L for the all-glass impinger. Statistical analysis indicated that the means were significantly different.

One experiment was conducted in the test chamber in which *S. marcescens* cells were disseminated by a generator developed by Arthur D. Little Company[6] which was capable of producing homogeneous aerosols below 3.0 μ MMD. Samples were taken at 1 to 2 min and at 6 to 7 min after dissemination with both the Shipe sampler and the all-glass impinger. Results of these comparisons are shown in table 3. The mean plate count for the Shipe sampler was 250,000 cells per L as compared to 200,000 cells per liter of aerosol for the all-glass impinger at the 1 to 2 min sampling period, and 160,000 cells per L as compared to 130,000 cells per L respectively for the 6 to 7 min sampling period.

A series of trials was conducted in the test chamber to compare the collection of four aerosol samplers, the all-glass impinger, the capillary impinger, the Midget impinger, and the Shipe sampler. A mixed suspension composed of cells of *S. marcescens* and *B. subtilis* spores was disseminated by DeVilbiss no. 40 nebulizers producing an aerosol of <3.0 μ MMD. Before dissemination, two 16-in. fans operated at low speed were placed on blocks approximately 2 ft from the aerosol generators so as to fan in an upward direction. The fans were started prior to generation of the aerosol and operated through the 2- to 3-min sampling period. Six nebulizers were connected in parallel, each containing 1.1 ml of cell suspension (1.2×10^9 per ml *S. marcescens* and 7×10^8 per ml *B. subtilis* spores). Air pressure of 25 psi was applied to the nebulizers for a period of 7 min

[4] The DeVilbiss Company, Toledo, Ohio.

[5] Standard deviation of the mean (standard error) unless otherwise indicated.

[6] Arthur D. Little Company, Cambridge, Massachusetts.

TABLE 1

Loss from exhaust of Shipe sampler of aerosol droplets of Bacillus subtilis spores in a typical trial

Plate Count per L Aerosol Sampled		Loss from Exhaust
Shipe sampler	Cotton sampler in tandem	
× 10^4	× 10^4	%
210	4.8	2.2
290	8.8	2.9
310	9.9	3.1
470	8.3	1.7
350	15.5	4.2
410	12.2	2.9

TABLE 2

Shipe sampler versus the all-glass impinger for recovery of small aerosol droplets of Serratia marcescens in the glass tube aerosol unit*

Trial	Plate Count per L of Aerosol Sampled	
	Shipe sampler	All-glass impinger
1	1,510,000	920,000
2	1,320,000	1,036,000
3	1,548,000	992,000
4	1,649,000	976,000
5	1,795,000	1,032,000
6	1,687,000	872,000
Mean	1,584,000	972,000

* Less than 3.0 μ MMD (mass median diameter) generated by a DeVilbiss no. 40 nebulizer. *S. marcescens* suspension control count 0.98×10^9 per ml.

TABLE 3

Shipe sampler versus the all-glass impinger for recovery of small aerosol droplets of Serratia marcescens in the test chamber

Sampling Period	Plate Count per L of Aerosol Sampled	
	Shipe sampler	All-glass impinger
1 to 2 min	291,000	224,000
	237,000	242,000
	232,000	107,000
	241,000	211,000
Mean	250,000	196,000
6 to 7 min	175,000	121,000
	135,000	132,000
	145,000	124,000
	164,000	138,000
Mean	155,000	129,000

S. marcescens suspension control count 30×10^8 per ml.

to accomplish dissemination of the cells. One set of each of the four samplers was operated at four sampling periods: 2 to 3, 4 to 5, 8 to 9, and 16 to 17 min after dissemination. A summary of the results obtained is shown in table 4. Analysis of the data revealed differences in recoveries for the four samplers. When *S. marcescens* was the test organism, the most effective samplers were the Shipe sampler, the Midget impinger, and the all-glass impinger with no differences detected among them. There was some indication that the above listing was in the order of decreasing effectiveness. The capillary impinger was significantly less efficient, collecting about 40 per cent as many as the others. When *B. subtilis* was the test organism, recoveries were not significantly different among the four samplers. However, it was noted that the Shipe sampler, which gave the highest recovery of *S. marcescens*, also gave the highest recovery of *B. subtilis*. The remaining samplers in order of decreasing recovery were the capillary impinger, the all-glass impinger, and the Midget impinger, which was the reverse order of that found with *S. marcescens*. The implication was that, with the exception of the Shipe sampler, the most effective sampler for *S. marcescens* was the least effective for *B. subtilis*.

Comparison of samplers for collection of aerosols of >3.0 μ MMD. Using the same samplers in a similar comparative study, aerosols of *S. marcescens* were generated in the test chamber by an explosive fixture containing 8.8 ml of approximately 2.5×10^9 cells per ml resulting in a heterogeneous aerosol. Six trials were conducted. A summary of the data is shown in table 4. When explosively disseminated clouds were sampled, the Shipe sampler recovered significantly more organisms at each sampling period than any of the other three samplers. There was no significant difference between the all-glass impinger and the capillary impinger for sampling the explosively disseminated clouds.

In conjunction with trials in which four variations of an explosive fixture were conducted in the test chamber, sampling studies were included to determine differences between the all-glass impinger and Shipe sampler under conditions in which there were variations in the disseminator. Shipe samplers were placed at each of four sampling stations and operated in parallel with all-glass impingers. Three replicates were obtained for each of the four fixture variations and at three sampling periods of 1 to 2, 6 to 7, and 18 to 19 min after dissemination. A few determinations of particle size were made by use of cascade impactors (May 1945), but were insufficient to establish the reliability of the measurements. Results of these comparisons are expressed as a ratio of the cell count obtained from the Shipe sampler to the cell count obtained from an all-glass impinger. The results are shown in table 5. The data indicated that the ratios between the two samplers decreased with a decrease in MMD of the aerosol.

The Venturi scrubber, as modified by the authors, was evaluated on a limited basis by comparison with the Shipe sampler and the all-glass impinger in the test chamber using an aerosol generated by an explosive fixture under conditions similar to those previously described. The data are presented in table 6. Again the Shipe sampler appeared to give better recovery than either the Venturi scrubber or the all-glass impinger.

TABLE 4

Recoveries in four samplers of small or heterogeneous aerosol droplets of Serratia marcescens and Bacillus subtilis spores

Aerosol	Type of Sampler	Plate Count per L Aerosol at Indicated Period (min):			
		2–3	4–5	8–9	16–17
S. marcescens in mixed aerosol (small droplets*)	Shipe sampler	4,700†	4,550	3,500	1,820
	Midget impinger	4,500	3,430	2,950	1,980
	All-glass impinger	3,780	3,250	2,060	1,710
	Capillary impinger	1,560	1,480	1,210	700
B. subtilis in mixed aerosol (small droplets)*	Shipe sampler	5,340	6,930	4,230	4,800
	Midget impinger	4,100	3,700	4,000	3,600
	All-glass impinger	5,440	4,780	4,230	4,280
	Capillary impinger	5,900	4,700	5,000	4,860
S. marcescens aerosol (heterogeneous droplets‡)	Shipe sampler	20,400	16,600	10,900	5,900
	Midget impinger	12,800	12,300	12,900	4,000
	All-glass impinger	6,800	5,400	4,000	2,800
	Capillary impinger	6,800	6,700	5,000	3,300

* Less than 3.0 μ MMD (mass median diameter), Devilbiss no. 40 nebulizer.

† Each value is mean of 5 samples. Suspension counts: mixed, *S. marcescens*, 11.7×10^8 per ml; *B. subtilis*, 7.1×10^8 per ml; *S. marcescens* only, 24.6×10^8 per ml.

‡ Greater than 3.0 μ MMD, explosive disseminator in test chamber.

Additional studies were not conducted due to the difficulty in handling and construction of this particular sampler.

Further studies were conducted using relatively large explosive disseminators in the test sphere. Comparisons were made between the Shipe sampler and the standard impinger for collection of aerosol. Samples were taken at 1.5 to 2.5, 8.5 to 9.5, and 17.5 to 18.5 min after dissemination. Two concentrations of cell suspensions were used as fill for the explosive device: 1×10^{11} and 1×10^{9} *S. marcescens* cells per ml. The results and comparisons of ratios are shown in table 7. In all instances, recovery was greater in the Shipe sampler. It should be noted that the ratio of Shipe sampler to standard impinger decreased with the length of time after dissemination. Data of this type indicated retention of larger particles within the intake tube of the standard impinger.

A series of studies was undertaken in which the above two samplers were compared for recovery of *E. coli* cells disseminated under similar conditions. Samples were taken at 1 to 2, 8 to 9, and 18 to 19 min after dissemination with samplers located in the top, at the equator, and in the well of the test sphere. These data are shown in table 7 and substantiated the findings with *S. marcescens*.

The Shipe sampler was compared to the all-glass impinger in field trials in which *S. marcescens* cells were disseminated from an explosive device on a field grid area characterized by sampling stations spaced on 50- and 100-yard arcs from the point of dissemination. Parallel samples were taken with both samplers at each station. Data from a typical trial are shown in table 8. From a total of 79 comparisons made on the 50-yard arc, the ratio of Shipe sampler to all-glass impinger

TABLE 5

Shipe sampler versus the all-glass impinger for recovery of heterogeneous aerosol droplets of Serratia marcescens explosively generated in the test chamber

Fixture	Ratio of Shipe Sampler to All-Glass Impinger at Indicated Time Periods (min)*			Estimated MMD at Time (min):†
	1–2	6–7	18–19	5–6
				μ
1	3.1 ± 0.8	1.8 ± 0.6	1.4 ± 0.6	4.7
2	3.0 ± 0.8	1.7 ± 0.6	2.7 ± 0.5	6.1
3	5.3 ± 1.9	4.0 ± 3.1	3.2 ± 1.9	12.2
4	5.2 ± 2.3	3.2 ± 1.2	2.4 ± 1.2	10.9

* Each ratio represents the mean and standard deviation of 10 to 12 samples.

† Reliability of these values is low. MMD = mass median diameter.

TABLE 6

Shipe sampler versus the all-glass impinger and the Venturi scrubber for recovery of heterogeneous aerosol droplets of Serratia marcescens explosively generated in the test chamber*

Trial	Plate Count per L of Aerosol Sampled		
	Shipe sampler	All-glass impinger	Venturi scrubber
1	690,000	131,000	810,000
2	660,000	130,000	430,000
3	691,000	64,000	420,000
4	1,220,000	1,020,000	811,000
5	480,000	800,000	551,000
6	410,000	62,000	320,000
Mean...	690,000	370,000	560,000

* Aerosol particles >3.0 μ MMD (mass median diameter) generated by an explosive fixture. All samples taken at the 1.5- to 2.5-min period.

TABLE 7

Comparison of Shipe sampler and standard impinger in heterogeneous aerosols of Serratia marcescens and Escherichia coli in the test sphere*

Test Organism	Sampling Location	Ratio of Recovery (Shipe per Standard) per L Air Sampled at Indicated Period (min):		
		2	9	18
S. marcescens	Bottom (*A*)†	13.6‡	7.8	5.6
	(*B*)	7.2	4.9	4.9
E. coli	Top	16.3 (±6.1)§	11.8 (±5.3)	7.1 (±3.4)
	Equator	12.1 (±4.5)	6.6 (±1.9)	4.1 (±1.4)
	Bottom	11.4 (±1.6)	6.2 (±2.0)	3.3 (±0.8)

* Droplets >3.0 μ MMD (mass median diameter), generated by explosive disseminator.

† Suspension counts: *A*, 1,000 × 10^8 per ml; *B*, 10 × 10^8 per ml.

‡ Each value is mean of 2 trials.

§ Each value is mean of 11 or 12 trials with standard deviation of the values.

TABLE 8

Shipe sampler versus the all-glass impinger for recovery of aerosol droplets of Serratia marcescens explosively generated in the field in a typical trial

Arc	Station	Plate Count per Unit Vol Aerosol Sampled		Ratio: Shipe Sampler/All-Glass Impinger
		Shipe sampler	All-glass impinger	
Trial 1 (50 yards)	6	550	608	0.9
	7	3,064	1,020	3.0
	8	5,331	3,720	1.4
	9	25,662	10,480	2.4
	10	19,298	8,000	2.4
	11	6,108	1,620	3.8
Trial 2 (100 yards)	8	2,358	1,144	2.1
	9	7,820	5,440	1.4
	10	6,710	5,080	1.3
	11	204	292	0.7

counts was 3.13 ± 3.25; and of 51 comparisons made on the 100-yard arc, the ratio was 2.43 ± 2.43.

Discussion

Data have been presented which demonstrate three points: The first was that recovery in the Shipe sampler was equal to or better than recovery in any of the samplers with which it was compared although its collection efficiency was slightly lower than that of the all-glass impinger. The second finding indicated a relationship between particle size and the ratio of recovery. Collection of cells from aerosols of $<3.0\ \mu$ MMD resulted in a lower margin of difference between the samplers, particularly when sampling aerosols of spores. The greater the particle size the greater was the difference between the Shipe sampler and those characterized by intake tubes. A third point was the fact that with homogeneous aerosols of $<3.0\ \mu$ MMD there appeared to be less difference between the Shipe sampler and the other impinger samplers when sampling aerosols of sporeforming organisms than of nonsporing bacteria. This suggests the susceptibility of the latter to certain impinging effects. The last two points were more thoroughly investigated and are reported separately (Tyler *et al.*, 1959).

Acknowledgments

Grateful acknowledgment is made of the technical assistance of Mr. Floyd Martin. Personnel of several technical groups at Fort Detrick are thanked for cooperation in permitting use of facilities in the study.

Summary

Development of the Shipe sampler has been described along with comparative studies on other types of aerosol samplers for collection of homogeneous and heterogeneous aerosols of *Serratia marcescens*, *Bacillus subtilis* spores, and *Escherichia coli*. Tests were performed in various small and large aerosol units, and in the field. In all cases the Shipe sampler collected at least as many organisms from homogeneous ($<3.0\ \mu$ mass median diameter (MMD)) aerosols as did any of the other samplers tested. Collection of aerosols of vegetative cells was greater with the Shipe sampler than with the other samplers tested. Studies with heterogeneous aerosols indicated that the ratio of recovery between the Shipe sampler and other impinger samplers increased with increase in MMD of aerosol particles.

REFERENCES

May, K. R. 1945 The cascade impactor, an instrument for sampling coarse aerosols. J. Sci. Instr. **22**, 187–195.

Tyler, M. E. and Shipe, E. L. 1959 Bacterial aerosol samplers. I. Development and evaluation of the all-glass impinger. Appl. Microbiol., **7**, 337–349.

Tyler, M. E., Shipe, E. L., and Painter, R. B. 1959 Bacterial aerosol samplers. III. Comparison of biological and physical effects in liquid impinger samplers. Appl. Microbiol., **7**, 355–362.