REFERENCES

EISMAN, P. C., MARSH, W. S., AND MAYER, R. L. 1946 Differentiation of antibiotics by resistant strains. Science, **103,** 673-674.

HARRIS, D. A. AND RUGER, M. L. 1953 Microbiological aspects of new antibiotics screening. I. A plug test procedure. Antibiotics & Chemotherapy, **3,** 265-270.

STANSLY, P. G. 1946 The presumptive identification of antibiotics. Science, **103,** 402-403.

STAPLEY, E. O. AND ORMOND, R. E. 1957 Similarity of albomycin and grisein. Science, **125,** 587-589.

SZYBALSKI, W. 1953 Genetic studies on microbial cross resistance to toxic agents. II. Cross resistance of *Micrococcus pyogenes* var. *aureus* to 34 antimicrobial drugs. Antibiotics & Chemotherapy, **3,** 915-918.

SZYBLASKI, W. 1954 Genetic studies on microbial cross resistance to toxic agents. IV. Cross resistance of *Bacillus megaterium* to 44 antimicrobial drugs. Appl. Microbiol., **2,** 57-63.

SZYBALSKI, W. AND BRYSON, V. 1952 Genetic studies on microbial cross resistance to toxic agents. I. Cross resistance of *Escherichia coli* to 15 antibiotics. J. Bacteriol., **64,** 489-499.

SZYBALSKI, W. AND BRYSON, V. 1954 Genetic studies on microbial cross resistance to toxic agents. III. Cross resistance of *Mycobacterium ranae* to 28 antimicrobial agents. Am. Rev. Tuberc., **69,** 267-279.

USDIN, E., SHOCKMAN, G. D., AND TOENNIES, G. 1954 Tetrazolium bioautography. Appl. Microbiol., **2,** 29-33.

Design and Evaluation of a Slit-Incubator Sampler[1]

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Research laboratories, hospitals, and civil defense agencies are in need of a simple sampling device for sensitive vegetative cells that will determine changes in air-borne bacterial contamination with a high degree of efficiency over a considerable period of time. Epidemiological studies of modes of transmission of air-borne disease would be simplified if more were known regarding the bacteriological content of the air in locales where disease has been transmitted by suspected air-borne routes. This is of extreme importance since several hospitals are currently reporting epidemics of infections by *Staphylococcus* sp. among patients and staff personnel. (Fekety *et al.*, 1958; Murray *et al.*, 1958)

Decker and Wilson (1954) have reported previously on a slit sampler for collection of air-borne microorganisms. This earlier sampler possessed inherent advantages over those developed by previous workers. However, its collection efficiency decreased if it was used continuously for periods of time much longer than 1 hr.

Recently, Kuehne and Decker (1957) have reported on several critical factors affecting the efficiency of air sampling when vegetative cells of microorganisms are collected for extended periods of time. Factors such as increasing the volume of air per given area of agar is detrimental to the viability of *Serratia marcescens*. Decreasing the agar concentration from 3 per cent to 1.5 per cent was also found to support greater growth of the test organism.

As a result of these recent studies on air sampling, a more efficient slit sampler has been designed, constructed, and evaluated. This sampler permits continuous collection of air-borne vegetative organisms for long periods with a much higher degree of efficiency by providing a larger area for collection of microorganisms than samplers previously developed by the authors and others. Collection of a much greater number of organisms with less dehydration of the media and rendering of an accurate time concentration relationship results. There is also incorporated within the sampler an incubation chamber which is automatically turned on at the completion of sampling, requiring attendance of the sampler only once in a 24-hr period.

MATERIALS AND METHODS

The slit-incubator sampler and its component parts are shown in figures 1 and 2. The sampler is composed of a stainless steel and lucite sampling box 40 in. long, 4 in. wide, and 4¾ in. high, a slit and slit tube, media culture tray, rotating drive shaft, electric clock motor, and heating element.

The slit and slit tube assembly is threaded into the sampling box. The slit opening may be adjusted by moving one of the metal plates which are fastened to the bottom of the slit tube. The height indicator, a vertical metal shaft passing through the sampler top, is temporarily set with its lower end on the media surface.

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The distance between the slit and the media is correct when the top surface of the outer rim of the slit tube is flush with the top surface of the height indicator. The indicator is then raised from the media surface. The metal sampling box has a plastic top and houses the rectangular culture tray and the incubation section. The rotating threaded drive shaft is motivated by an electric clock motor.

To utilize the sampler, a sterile rectangular tray filled with a selective growth medium is inserted through an opening and is engaged on the threaded shaft by means of a metal clip. The opening is then sealed by an airtight door. The shaft pushes the tray forward underneath the slit at a fixed rate for the sampling period. Air is drawn through the slit at a rate of 0.5 cubic foot per min. (cfm) by means of a suitable vacuum. The width of the slit is set at 0.004 in., resulting in an air velocity of 110 feet per second. The air-borne microorganisms are impinged on the surface of the passing medium and the portion of the tray that has already passed under the slit enters the incubation section. After sampling is complete and the entire tray has entered the incubator section, the rotating shaft and motor are stopped by a microswitch and the incubator is then turned on. Heat is maintained by a heating tape and the temperature is kept constant by a thermostat. The tray can be removed through the door before incubation, if desired.

The efficiency of the slit-incubator sampler was determined in accordance with a statistical test plan (randomized block design). *S. marcescens* was used as the test organism and the organisms were aerosolized in a 1500 L plexiglas chamber at a temperature of 20 to 25 C and a relative humidity of 40 to 50 per cent. A dynamic aerosol was maintained by atomizing a suspension of *S. marcescens* with a Vaponefrin[3] nebulizer. Eighty per cent of the particles produced were less than 2 μ in size. Two types of samplers were used: a 1-hr round slit sampler operating at 1 cfm (Kuehne and Decker, 1957) and the slit-incubator sampler operating at ½ cfm. The round slit sampler is shown in figure 3. In both cases, the slit opening was 2.0 mm above the surface of the agar and the organisms were collected on Wilson's[4] peptone medium containing 1.5 per cent agar.

[3] Vaponefrin Company, Upper Darby, Pennsylvania.
[4] Wilson and Company, Chicago, Illinois.

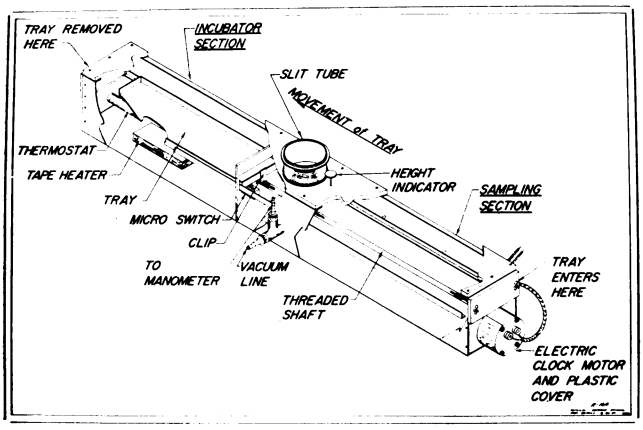

Figure 1. Slit-incubator sampler

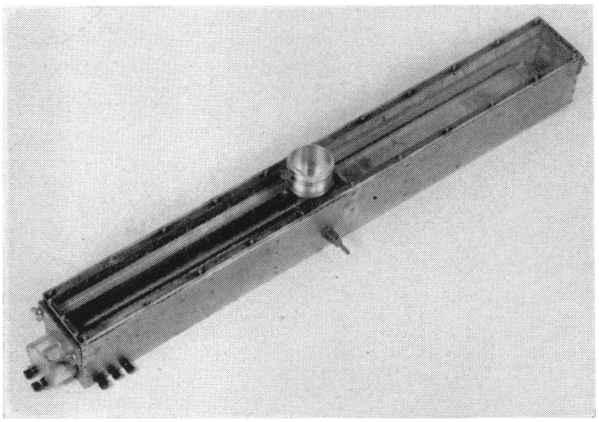

Figure 2. Slit-incubator sampler

Figure 3. One-hour round slit sampler

TABLE 1

Recovery of Serratia marcescens using the slit-incubator sampler

Speed Setting*	Total Time of Sampling	No. of Trials	Mean Hourly Recovery†	95 Per Cent Confidence Limits
hr	*hr*		%	%
12	1	21	59.0	52.2–66.7
12	5.5	18	57.4	49.9–66.1
12	11	18	32.4	28.2–37.3
7.5	1	11	74.0	65.3–81.7
7.5	7	30	74.0	55.8–92.1

* Time required for passage of entire length of tray under the slit.

† Based on a 1-hr round slit sampler as unity.

RESULTS

Tests were first made to determine the efficiency of a 12-hr slit-incubator sampler when sampling for 1-hr periods only. The efficiency of the slit-incubator sampler was obtained by considering the average number of organisms collected per cubic foot of air sampled with a round slit sampler (making one complete revolution per hr) as unity and comparing this number with that collected with the slit-incubator sampler. The results of 21 trials (table 1) show that operating the sampler for 1 hr gives an average recovery of 59.0 per cent of a 1-hr round slit sampler. The analysis of these data did not indicate the presence of appreciable sampling variation.

Evaluation was then commenced, sampling continuously for 5.5 hr with a 12-hr slit-incubator sampler and determining the per cent recovery for the first hr and last hr of sampling (4.5 to 5.5 hr) using the 1-hr round slit samplers as controls. These results (table 1) show that, when sampling is done for 5.5 hr, an average of 57.4 per cent of the organisms are collected per hr when compared to the 1-hr round slit sampler and this recovery does not differ significantly from the first hr of sampling to the last.

The slit-incubator sampler was then run for 11 hr and the per cent recovery determined in the same manner for the first (0 to 1 hr) and last (10 to 11) hr of sampling. The results (table 1) show that when the sampler is used for 11 hr an average of 32.4 per cent of the organisms are collected every hr of the sampling period, based on a 1-hr round slit sampler as unity. Again the recoveries of the first and last hr of sampling do not differ significantly. This recovery is twice the recovery reported by Kuehne and Decker (1957) when a round slit sampler is used for 12 hr.

It is interesting to note that, when the sampler is used for 5.5 hr, the same recovery per hr is obtained as when it is run for only 1 hr. However, somewhere between 5.5 and 11 hr, a decrease in growth occurs, not merely of the organisms collected on the first hr of sampling, but also of the last hr.

Therefore it was believed that a 7 to 8 hr sampler would give a higher efficiency and would have practical application, as this time simulates that of a working day. A sampler was modified to run 7.5 hr by replacing the motor with a faster one and evaluated as above. This change resulted in greater agar surface area per unit of time for collection of the organisms. When this sampler is operated for 7 hr, an average of 74.0 per cent of the organisms are collected per hr, using the 1-hr round sampler as unity (table 1) and the recovery is again constant during the entire sampling period. When it is used for only 1 hr, the hourly recovery is the same as when operated for 7 hr.

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SUMMARY

A sampler has been designed which permits continuous sampling of vegetative organisms with a high degree of efficiency. The organisms in the air are collected on a selective agar in a stainless steel tray which passes under a slit opening located in the top of the sampler. A unique feature is the incorporation of an incubator chamber which is automatically turned on at the completion of sampling, requiring attendance of the sampler only once in a 24-hr period. Civilian defense agencies, hospitals, and research laboratories can utilize this sampler for time-concentration detection of microoganisms in the atmosphere. Air-borne transmission of disease over long time periods should be traced more readily by the use of this sampler.

REFERENCES

DECKER, H. M. AND WILSON, M. E. 1954 A slit sampler for collecting air-borne microorganisms. Appl. Microbiol., **2**, 267–269.

FEKETY, R. F., BUCHBINDER, L., SHAFFER, E. L., GOLDBERG, S., PRICE, H. P., AND PYLE, L. A. 1958 Control of an outbreak of staphylococcal infections among mothers and infants in a suburban hospital. Am. J. Public Health, **48**, 298–310.

KUEHNE, R. W. AND DECKER, H. M. 1957 Studies on continuous sampling of *Serratia marcescens* using a slit sampler. Appl. Microbiol., **5**, 321–323.

MURRAY, W. A., JR., MCDANIEL, G. E., AND REED, M. 1958 Evaluation of the phone survey in an outbreak of staphylococcal infections in a hospital nursery for the newborn. Am. J. Public Health, **48**, 310–318.