A SLIT SAMPLER FOR COLLECTING AND COUNTING AIR-BORNE BACTERIA

BY R. B. BOURDILLON, O. M. LIDWELL AND JOHN C. THOMAS

From the National Institute for Medical Research, London, N.W. 3, and a Military Laboratory

(With Plates II–IV and 5 Figures in the Text)

CONTENTS

	PAGE
1. Methods of counting air-borne bacteria	198
2. The slit sampler	199
Description	199
Method of use	202
Precautions	203
Sterilization	203
3. Methods of testing	203
4. Efficiency tests	205
Definitions	205
Methods of determining efficiency	205
(1) Use of two similar samplers in tandem	205
Limitations of tests with two similar samplers in tandem	206
(2) Dissimilar samplers in tandem	206
(3) Use of a sampler of one type in air, before and after passing a sampler under test, which may be of the same type or dissimilar	207
(4) Comparison of two different samplers in parallel	207
5. Results	207
Uniformity of sampling	207
Collection efficiency	208
(*a*) Effect of particle size, and of slit-plate distance	208
(*b*) Effect of rate of flow of air	210
(*c*) Effect of humidity	210
(*d*) Effect of size and shape of slit	211
6. Direct comparison with other methods	212
The Wells's centrifuge	212
The funnel method	214
McConnell and Thomas's aeroscope	214
Sedimentation on open Petri dishes with agar jelly	214
7. Tests for possible sources of inefficiency	217
(1) Death from concussion	217
(2) Overdrying on prolonged suction	218
(3) Adhesion of organisms to entry tube or walls of slit	218
(4) Length of incubation period	218
8. Alternative methods of using slit sampler	219
9. Discussion	219
Acknowledgements	220
Summary	221
Appendix. 1. Constructional details	221
2. Samplers in tandem and heterogeneous atmospheres	223
References	224

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1. Methods of counting air-borne bacteria

The current methods of collecting and counting bacteria from samples of air suffer either from doubtful efficiency or from the need for such care in use that the taking of numerous samples becomes very laborious. Useful reviews of these numerous methods have been published by Ruehle (1915), Hahn (1929), and by the American Committee on Apparatus in Aerobiology (1941).

The chief methods may be grouped as follows:

(1) *Collection of bacteria on porous solid filters.*

The best known of such filters is sand, which has been used by many workers, but needs great care if high efficiency is to be obtained (Ruehle). Other workers use soluble salts; these, however, often cause bacterial inhibition, and prolong the subsequent incubation period up to 9 days (Oesterle, 1934). Sintered glass has been recommended by Hettche & Schwab (1940). This collects efficiently but requires very thorough washing. Cotton-wool also collects efficiently but is difficult to separate completely from the bacteria (Robertson & Doyle, 1940).

(2) *Collection of bacteria in a fluid medium.*

These methods either involve simple sedimentation (Ruehle; Pressman, 1937), or the bubbling of air through a column of liquid. The best known is perhaps the 'Aeroscope' of Rettger (1910), which has the drawback of needing very thorough washing of the air inlet tube (Ruehle). Some authors in describing methods of this type have paid too little attention to the well-known difficulty of extracting small solid particles from a gas by bubbling through a liquid. This difficulty is largely overcome by McConnell & Thomas (1925), who use a layer of Japanese silk immersed in the liquid of their Aeroscope. Such fine mesh silk when wet acts as a fairly efficient filter, but we have found it difficult to cultivate the organisms thus collected.

The above methods are all subject to the serious errors involved in plating out bacteria from a fluid medium, and they involve considerable work if these errors are to be kept small. They also need full precautions against contamination. Most of them split up to a greater or less extent such bacterial clusters as may be present, and thus give counts intermediate between the number of bacteria-carrying particles and the total number of bacteria present in a given volume of air.

(3) *Collection of bacteria on a solid culture-medium.*

These methods count only the bacteria-carrying particles in the air, without splitting up clusters. They are free from the errors of plating out liquid suspensions, and are much more rapid and convenient in use than those of the former group, although some of them are efficient for large particles only. They include the natural sedimentation of organisms on to a solid culture

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medium in an open Petri dish, and the collection of organisms by impact from a moving air stream as used by Hirvisalo (1933), by Hollaender & Dalla Valle (1939) in their funnel method, and by Wells (1937) in his air centrifuge. Although the Wells centrifuge has many advantages over most other instruments for air sampling, it demands considerable care in the preparation and counting of its culture tubes. We have endeavoured to avoid this and other drawbacks in designing the slit sampler described below.

2. THE SLIT SAMPLER

Owens (1922) described a jet dust-counter in which the dust particles were counted on a glass plate on which they had been collected by deposition from a jet of air moving at high velocity through a narrow slit placed 1 mm. from the glass plate. This instrument has been widely used for collecting and counting samples of atmospheric dust. Although Owens suggested its use for air-borne bacteria, his instrument was not well suited for such work and we have not seen any account of such adaptations as bacterial sampling requires. We have adopted Owens's principle, but have had to develop an instrument very different from his counter.

Description

In our slit sampler air is sucked through a narrow slit 0·25 mm. wide, 27·5 mm. long and 3 mm. deep on to the surface of agar or other culture medium contained in a Petri dish placed just below the slit. If the Petri dish were stationary during suction all the bacteria collected would be deposited in a narrow line and the colonies would grow too close for counting, as shown in Pl. III *a*. Therefore the Petri dish is slowly rotated during sampling so that the colonies are distributed uniformly as shown in Pl. III *b*. The slit is placed radial to the circular surface of the Petri dish with inner end 9 mm. from the vertical line through the centre of the dish. Hence the bacteria are deposited in an annular ring leaving a clear space of about 18 mm. diameter in the centre, Pl. IV *a* and *b*.

The Petri dish can be rotated either by hand or mechanically, since the speed of rotation is immaterial within wide limits: a sampler of each type is described. The simple hand-rotated model can be constructed by anyone accustomed to metal work, without the use of a lathe or milling machine (Pl. II; Figs. 1 and 2*a*). The motor-driven model needs screw-cutting tools for its construction, but is more convenient in use, especially when large numbers of tests are required (Pl. II; Figs. 1 and 2*b*). The body of the slit sampler is the same in both models (Fig. 1). It consists of an airtight metal box (1) with a large window in front closed during sampling by a wide strip of adhesive plaster which surrounds a glass window (Pl. II). The Petri dish is carried by a rubber covered disk (2) which is rotated by the spindle (3) passing through the plain tubular bearing (14). In the roof of the box is a removable metal tube (4) to the lower end of which are soldered two metal

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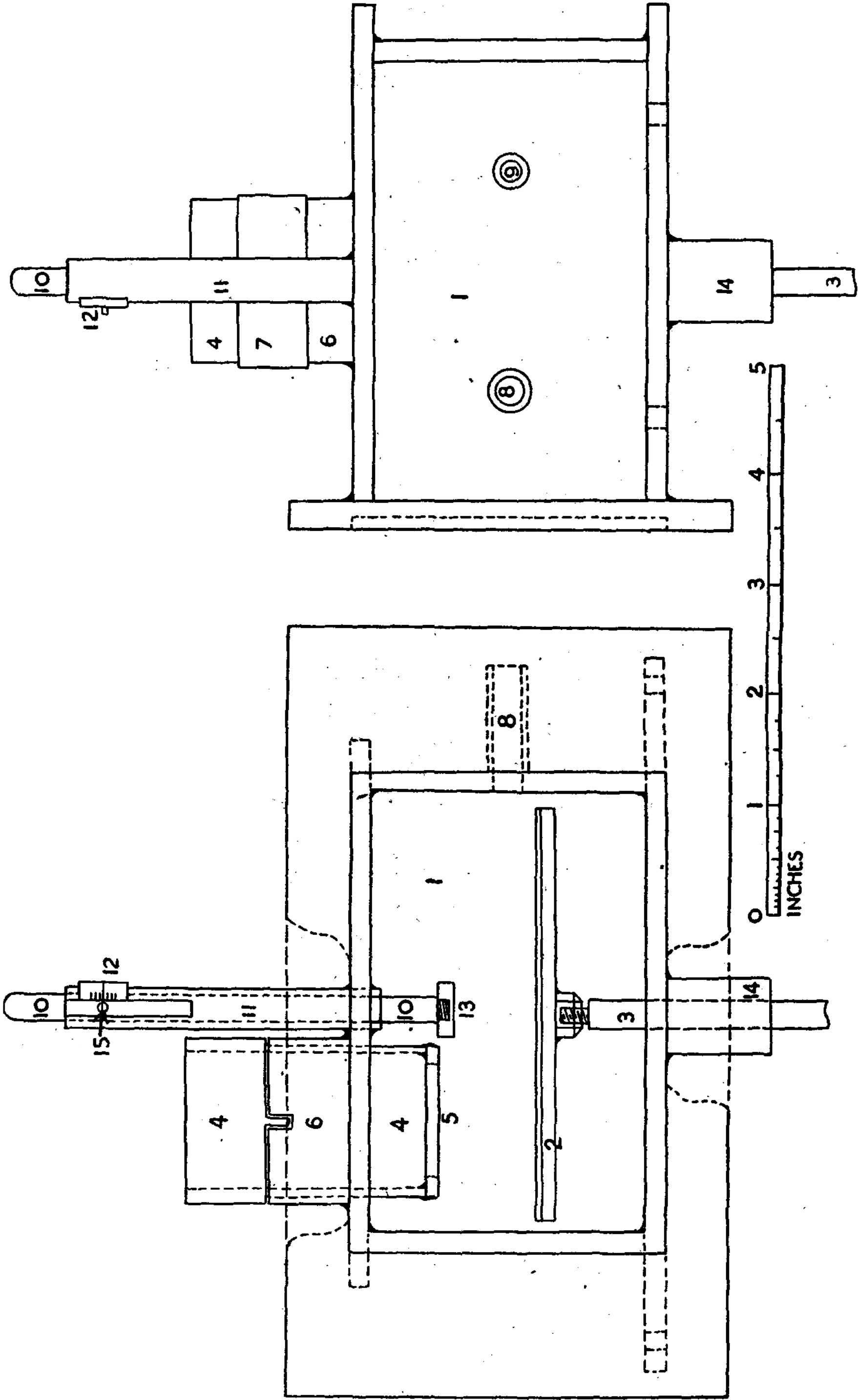

Fig. 1. The slit sampler. Body.

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jaws (5) forming the slit. There are two side outlets from the box, one (8) connected to a simple water manometer (Pl. II *b*), and the other (9) to a suitable power-driven suction pump with an adjustable by-pass valve in the connecting tube. When in use the suction causes a pressure difference inside the box depending on the rate of air flow through the slit. The by-pass valve is adjusted till the water column in the manometer reaches a mark fixed at 11 in. above the reservoir. Under these conditions air will flow through the slit at the rate of 1 cu. ft./min., which is the rate recommended for general use with a slit of the dimensions given.

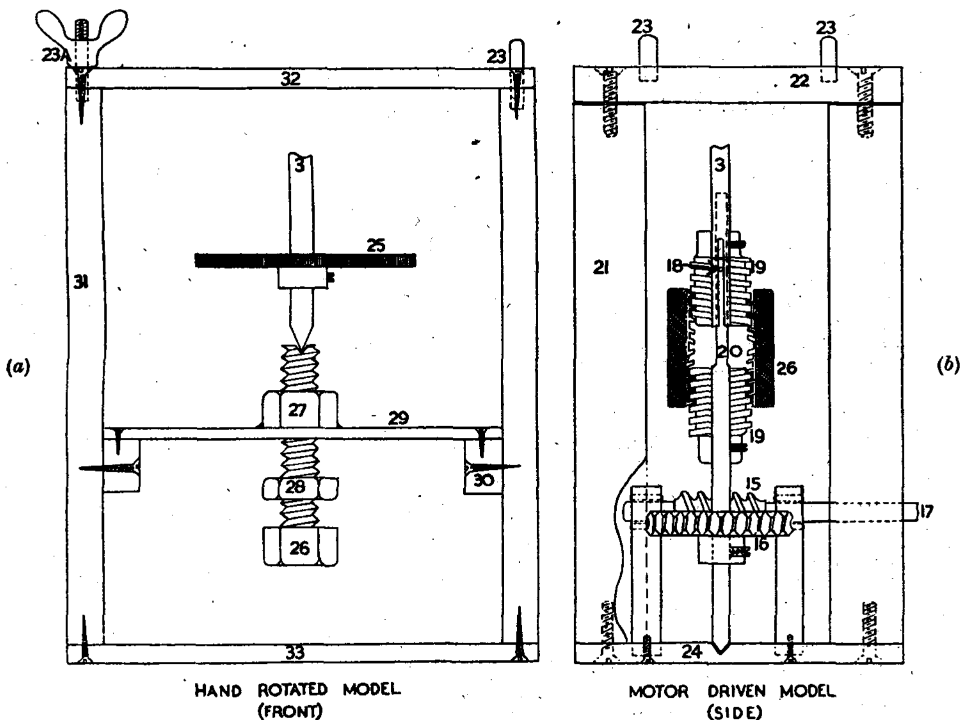

Fig. 2. The slit sampler. Stand. (*a*) For hand rotation. (*b*) Motor-driven.

As will be shown later the efficiency of the instrument varies with the distance between the slit and the surface of the culture medium which we call 'slit-plate distance'.

In our earlier experiments we used machines with a fixed slit-plate distance of 20 mm., as this allowed the plates to be inserted easily, and gave a sufficiently high efficiency for counting organisms as usually found in dust (Thomas, 1941; Thomas & van den Ende, 1941). We found later that this distance was too great for the efficient collection of single isolated bacteria, and so have now reduced it to 2 mm. To achieve this purpose the spindle is fitted with a height-adjusting device (Fig. 2, 26) so that the Petri dish can be raised when in use and lowered before insertion or removal. The correct height of 2 mm. from slit

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to agar surface is indicated by the scale (Fig. 1, 12) and indicator pin (15). This pin moves up and down with the rod (10) which slides freely in the tube 11 and ends in a brass disk (13). When the Petri dish is raised by the screw motion, at a certain height the centre of the agar surface touches the brass disk. As the dish is raised further the agar pushes up the disk and the indicating line on the pin moves up the scale to a red line indicating the 2 mm. distance. This makes it easy to adjust the height correctly within ½ mm. or less. Since the centre portion of the agar surface does not collect bacteria from the slit, and is not used for counting, no harm is done if an occasional contaminant does come from the brass disk. During suction the rod is pulled up and rotated slightly so that the indicator pin rests on the rim of tube 11, and thus prevents the disk 13 from falling back on to the plate. The constructional details of the samplers are given in the Appendix (section 1).

Method of use

The sampler is connected to the suction pump by a rubber tube with a T-piece of which one limb acts as a by-pass to the air of the room. The flow through this limb is controlled by a screw clamp on a piece of rubber tubing. (This can be seen in Pl. II *b* in front of the manometer reservoir.) When first starting a day's work it is advisable to have this clamp wide open. The pump is then switched on and the clamp tightened until the water in the manometer rises to the 11 in. mark. While actually sampling, a little further adjustment of the clamp is often desirable, as the pump performance may vary. As the manometer indicates the pressure difference across the slit, a moderate leak in most parts of the apparatus will not cause an error, since the experimenter will notice a lowering of the manometer column and will tighten the by-pass clamp until the normal pressure is restored. A variation of $\pm \frac{1}{4}$ in. of water height represents about 2 % of the volume of air passed.

To change Petri dishes the 'window' is opened by pulling the plaster fully to one side (Pl. II *a*) and the height-adjusting screw is turned until the rim of the dish is well below the slit jaws. The plate is then replaced by a fresh one, the height again adjusted, and the window resealed. In the motor-driven models the raising device is self-locking, but in the simple model the locknut (Fig. 2*a*, 28) should be tightened to prevent accidental alterations of height during the suction period.

The duration of suction should vary according to the state of the air under test. For a crowded room or canteen 2 min. (i.e. 2 cu. ft.) usually gives a satisfactory number of colonies on blood agar after 24 hr. incubation. In quiet hospital wards 5 cu. ft. is usually better. If gentian violet blood agar is used, about 10 min. suction (=10 cu. ft.) will be found best.

Even in very pure air suction should not be continued on any one plate for longer than 1 hr., for fear of overdrying the agar.

For a general sampling of the air of a room the most useful height of the

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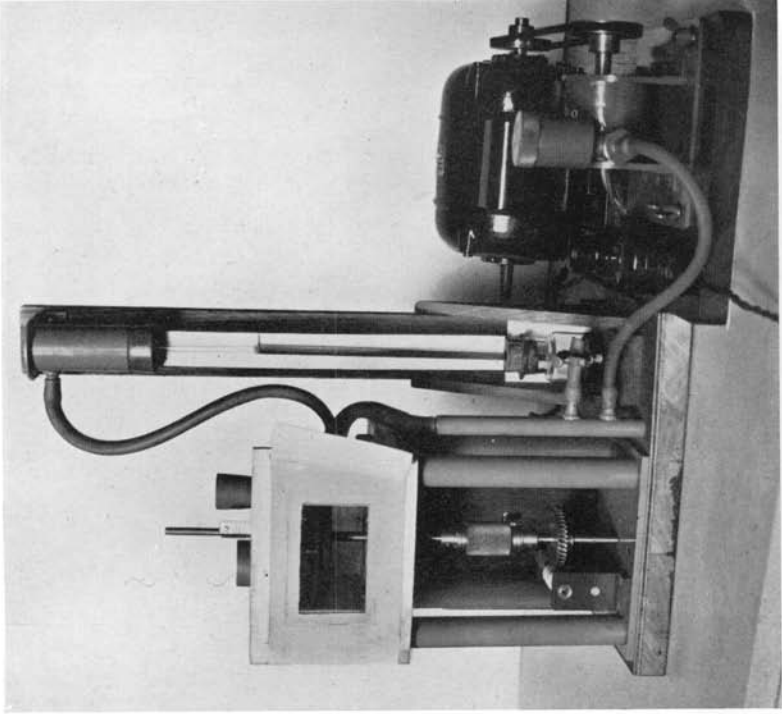
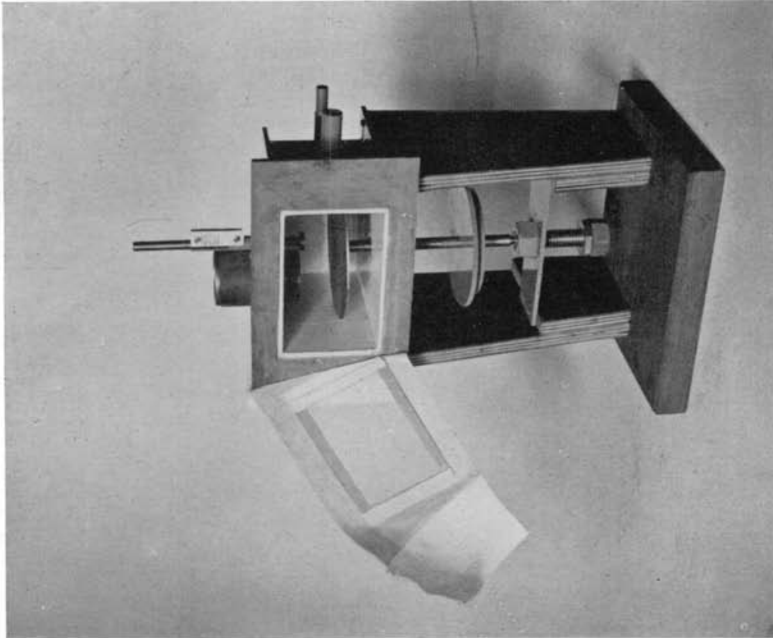
The slit sampler



(b) Motor-driven model in action.



(a) Hand rotated model.

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slit is either 3 ft. 6 in. or 5 ft. 3 in., corresponding to an average sitting or standing height of the human mouth and nose.

Precautions

1. When sampling it is important to avoid brisk movements that might liberate dust close to the slit, and especially to avoid bringing the hands over the slit or talking close to it. Otherwise the experimenter may collect many bacteria from his own person or clothes. This precaution is needed in all methods of sampling.

2. The slit should be cleaned at the beginning of each day's work by wiping the upper side with a dry cloth (or, if necessary, by washing with hot water followed by acetone or alcohol) and then inserting the edge of a piece of paper between the jaws and moving it a few times from end to end of the slit. Unless this precaution is taken, misleading results will be obtained by the gradual obstruction of the slit by wool fibres and other large particles.

3. The brass disk of the height indicator should be wiped with alcohol once daily.

Sterilization

When using these samplers with solid media we have usually found that sterilization was not essential, provided that the slit was cleaned daily. As a further precaution the inside of the box may be coated with a thin film of vaseline or medicinal paraffin to hold any bacteria that may settle on it. The slit jaws must not be oily for fear of undue adhesion of particles to the walls.

If, however, the sampler is used to determine total bacteria by replacing the agar medium by an adhesive liquid which is subsequently plated out, or if it is used to detect small numbers of pathogens soon after working in highly contaminated atmospheres, sterilization becomes important.

For this purpose the rubber connexions and adhesive plaster are removed, the wing nut (Fig. 2, 23 A) unscrewed and then the box is lifted off the pins (23). After unscrewing the spindle from the disk (2) the box can be placed in any sterilizer.

3. METHODS OF TESTING

In comparing different methods of air sampling, it is important to remember that most methods are more efficient in collecting large particles than small ones, and that a method may be highly efficient with a coarse, wet spray of large droplets, yet almost useless with single dry bacteria. We have therefore in addition to field trials on the air of hospitals, canteens, etc., done a long series of laboratory tests on finely sprayed cultures of bacteria under the following conditions.

Spray room. 3200 cu. ft. capacity, without artificial heating, and with door and windows closed. During tests a 12 in. electric fan was kept running with axis horizontal about 3 ft. above the floor. This caused a vigorous circulation of air in the room, and resulted in consecutive or adjacent samples of air showing good uniformity of bacterial content.

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Sprays

Coarse. A cheap nasal spray worked by a hand bulb was used to give a coarse wet spray. To avoid excessive sedimentation, which is rapid with this type of mist, this spray was used in a closed, vertical duct (36 × 15 × 15 in.; approximately 5 cu. ft.) and samples were taken 30 sec. after distribution.

Medium. An Atmozon spray (Messrs C. Hearson Ltd.), as sold for oral or nasal use, was supplied with air at 10 lb. pressure. This gives a finer spray than most commercial units, but as shown in Pl. IV *c* allows streptococci (*Strep. salivarius*) to pass in chains of fair length.

Fine. A spray of the Collison Inhaler type (Inhalation Institute Ltd., London), highly baffled and with a jet of 0·8 mm. diameter, was found to give a very fine spray from which 90 % or more of the bacteria-carrying droplets contained only single or diploid streptococci (Pl. IV *d*). Dr W. J. Elford kindly informed us that his tests showed a similar result with *Staph. albus.* This spray was used with 20 lb. air pressure per sq. in.

Cultures sprayed

We have most often used 18 hr. broth or serum broth cultures of the following organisms: *Streptococcus salivarius*, *Staphylococcus albus*, *Chromobacterium prodigiosum*, *Bacillus subtilis*, and Coccus 'A'—a large saprophytic coccus of about 1·5 μ diameter kindly given us by the National Collection of Type Cultures. It is difficult to estimate the size of dried particle from such cultures owing to uncertainty as to the volume of evaporated broth surrounding each organism. Therefore when the finest particles were required we used suspensions either taken off an overnight agar slope culture into a considerable volume of distilled water, or from a broth culture which had been washed twice, and taken up in distilled water.

We believe that the particles obtained with the fine spray from these suspensions *in water* were, after evaporation, not much larger than the single or diploid organisms which they contained—say 1 μ for *Staph. albus*, and 2 × 1 μ for diploid *Strep. salivarius*. Evaporation of nearly all the water from the spray droplets should occur within 1 sec. (Whytlaw-Gray & Patterson, 1932), and we found no change in efficiency when comparing samples taken directly after spraying with those taken 20 min. later, thus confirming our belief in such rapid evaporation.

Culture methods

For laboratory trials on sprayed cultures we have used plates containing 2 % agar in Hartley broth plus 10 % horse serum. For field trials we have used a similar agar with 10 % horse blood with, in certain cases, the addition of 1/500,000 gentian violet. Garrod (1933) showed that the latter medium was a useful differential medium for haemolytic streptococci, and Thomas, using it for aerial counts, showed that the aerial saprophytes were reduced

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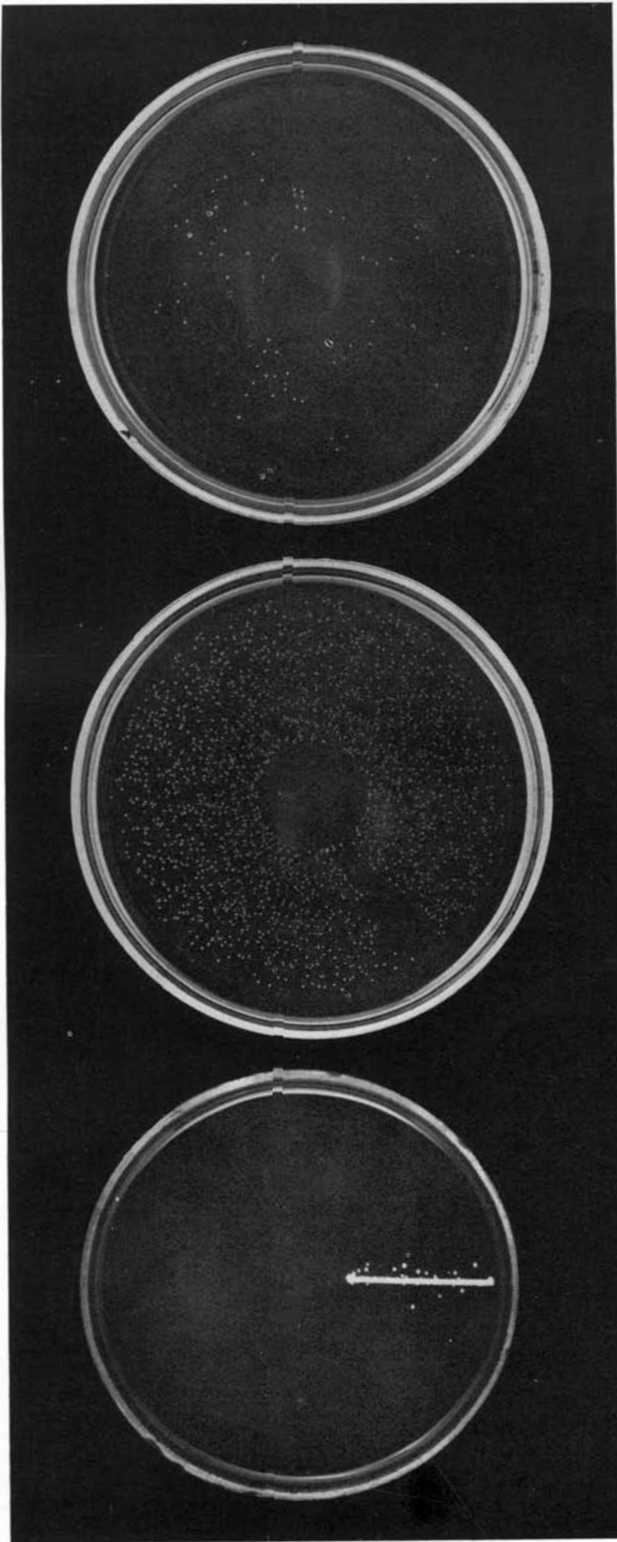

(*a*) 3000 colonies from sample taken with plate stationary.

(*b*) 1850 colonies, *Strep. salivarius*, on rotated plate.

(*c*) 59 colonies from a sampler in series with that used for (*b*).

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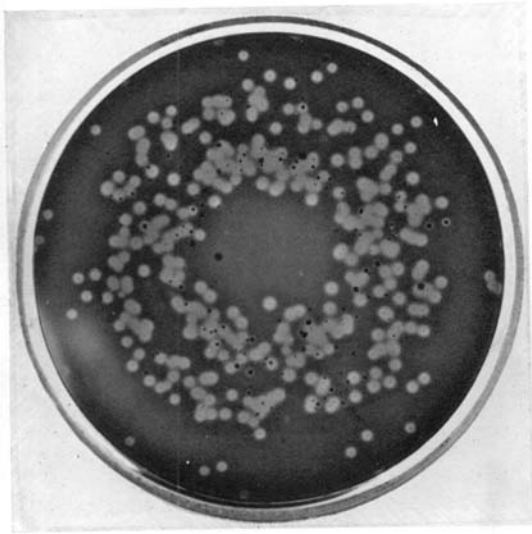
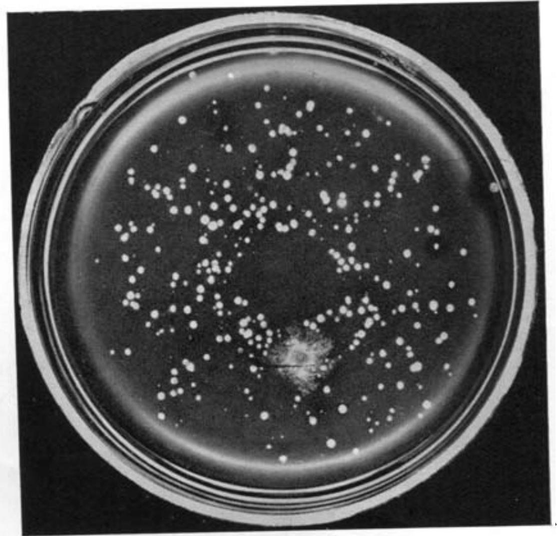
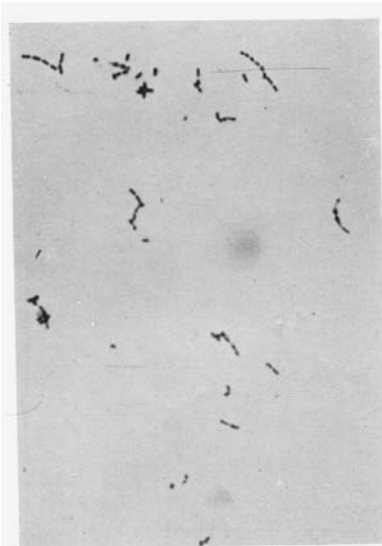


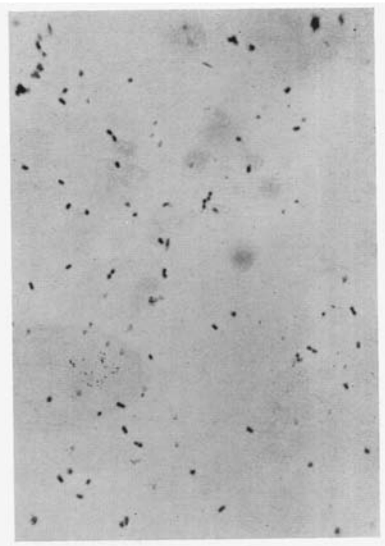
(*a*) β haemolytic streptococci from air of hospital ward during bedmaking. 2 cu. ft. 1/500,000 gentian violet blood agar. Photo. by transmitted light.



(*b*) From air of crowded canteen. 1 cu. ft. Blood agar. Photo. by reflected light.



(*c*)



(*d*)

(*c*) and (*d*) *Strep. salivarius* × 970 from aerosol in test spray room. As obtained by sucking air on to a plain glass slide in the sampler. (*c*) Chains of various lengths from the medium spray. (*d*) Single and diploid cocci from the fine spray.

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by 95 % without serious inhibition of pathogenic streptococci. Plates were counted after 18–24 hr. aerobic incubation, except in tests on the effect of incubation period on numbers of colonies, in which counts were made at intervals up to 72 hr.

4. Efficiency tests

Definitions

(*a*) *Viable bacteria.* We define as 'viable' all those bacteria which would grow to visible colonies if given the best possible environment for their particular species. This definition renders the determination of their number an ideal that can be approached rather than achieved.

(*b*) *Bacteria-carrying particles.* In the following sections, except 8 (1), we consider each particle carrying viable bacteria as one unit, without regard to the number of bacteria any one particle may carry, and, where apposite, shall use the term 'bacteria' to mean 'particles carrying viable bacteria'.

(*c*) *Collection efficiency.* Most methods of sampling divide the air-borne bacteria in each sample into three groups, namely:

A. Those which are collected and counted.

B. Those which are collected but fail to be counted.

C. Those which are not collected.

We define *collection efficiency* as the ratio of bacteria collected (both counted and uncounted) to total bacteria present in the sample of air, i.e. as

$$(A+B)/(A+B+C).$$

(*d*) *Estimation efficiency.* We define this as the ratio of bacteria collected and counted to total bacteria, i.e. as

$$A/(A+B+C).$$

Methods of determining efficiency

(1) *Use of two similar samplers in tandem.*

When two samplers are joined in tandem the second instrument samples the organisms which are not collected by the first. This method has been used by many workers, often without full recognition of the influence of size of particle on the results, or of some of the limitations discussed below. For a sample containing particles of uniform size the following equation is true, provided that no particles are deposited between the two sampling machines.

Let N = total organisms in the sample, and N_1 and N_2 = the organisms collected and counted in the first and second machines respectively.

Let $\alpha = A/(A+B+C)$, i.e. the fraction of the entering organisms that is collected and counted, and $\beta = B/(A+B+C)$, i.e. the fraction of the entrants that is collected but not counted.

Then $N_1 = N\alpha$.

The number of organisms entering the second sampler is $N(1-\alpha-\beta)$.

Of these a fraction α is counted.

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Therefore $N_2 = N\alpha(1-\alpha-\beta)$.

Hence
$$\frac{N_1-N_2}{N_1}=\frac{N\alpha-N\alpha(1-\alpha-\beta)}{N\alpha}=\alpha+\beta.$$

Now $\alpha+\beta=(A+B)/(A+B+C)$, i.e. the collection efficiency as defined above.

Thus the collection efficiency is equal to the difference between the counts in the first and second machines, divided by the first count, and this is true whether a large or a small fraction of the organisms fail to be counted, provided that the two samplers are similar.

It is often convenient to express collection efficiency as a percentage by multiplying $(N_1-N_2)/N_1$ by 100.

The results of such a tandem test with 2 mm. slit-agar distance are shown in Pl. III *b* and *c*. The number of colonies collected in the first sampler (*b*) was 1850, in the second (*c*), 59. Hence the collection efficiency was $(1850-59)/1850=0{\cdot}968$ or 96·8 %.[1]

Limitations of tests with two similar samplers in tandem. (*a*) The method becomes inaccurate when the collection efficiency is 20 % or less, owing to the small differences between N_1 and N_2. In practice this trouble is easily avoided by using a high efficiency sampler for the second machine, as described in method (2) below.

(*b*) *Effect of non-uniform particle size.* When a sampler is inefficient for the smaller particles present in an atmosphere, the above method can become very misleading. It may for instance show a high efficiency on large particles and yet fail to record any small particles at all. In a less extreme case the method will still tend to give an apparent collection efficiency higher than the true one, because the second sampler will receive an undue proportion of the smaller particles. It will therefore record too small a fraction of the particles that it receives; i.e. N_2 will be too small and $(N_1-N_2)/N_1$ will be larger than it should be. Strict proof of this is given in the Appendix (section 2).

It is evident that this source of error will be of especial importance in sampling atmospheres in which the number of particles increases as the size decreases, e.g. in certain types of dust sampling. We believe it to be quite unimportant in the slit sampler (when used for sampling bacteria), owing to the high efficiency of this machine for single isolated bacteria, i.e. for the smallest particles concerned.

(2) *Dissimilar samplers in tandem.*

A sampler of unknown efficiency followed by a sampler of known high efficiency in tandem.

This method is very useful for testing samplers of low efficiency provided that the numbers collected but not counted can be assumed to be small, or

[1] The two samplers were connected by sweating a tube of 1 in. bore into the top plate of the front sampler and joining this by wide-bore glass tubing to the slit tube of the second sampler. The rate of flow was measured by the first manometer, the second being shut off.

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else that the estimation efficiency of the second sampler is known. In such a case N_2 divided by the known efficiency E_2 of the second sampler gives the number of organisms leaving the first sampler. Hence the collection efficiency of the first sampler $E_1 = N_1/(N_1 + N_2/E_2)$.

(3) *Use of a sampler of one type in air, before and after passing a sampler under test, which may be of the same type or dissimilar.*

For example, if a sampler of type *A* collects 100 organisms from air before it passes through sampler *B* and only 10 organisms after the air has passed *B*, the collection efficiency of *B* is 0·9 or 90 %. Provided that the particle size is uniform, this method can give a reliable estimate of the collection efficiency of *B* without requiring any knowledge of the efficiency of sampler *A*.

(4) *Comparison of two different samplers in parallel.*

This method—simultaneous sampling by two adjacent machines—has been widely used, and is very valuable provided that the particle distribution is uniform in the air from which samples are taken. It measures the ratio of the estimation efficiencies of the two samplers for the test particles concerned, but gives no absolute measure of *estimation* efficiency unless that is already known for one of the two samplers.

5. Results

Uniformity of sampling

In tests that are made by taking successive samples from a given atmosphere there will be three sources of error: (1) The random error of samples containing a finite number of discrete particles. This error is such that a series of samples taken by a perfect instrument from a uniform atmosphere for which the true mean number per sample is M should show a standard deviation from this mean of $\sqrt{M}$ or, expressed in percentage terms, a coefficient of variation of $100/\sqrt{M}$. (2) Errors due to non-uniformity (i.e. imperfect mixing) in the atmosphere. (3) Errors due to the sampling technique itself.

Our tests of sampling have not been numerous enough to give a close estimate of their total errors. We have, however, frequently obtained series such as those shown in Table 1. These series are surprisingly uniform, and indeed, in each case, the observed coefficient of variation is less than that to be expected from the errors of random sampling alone ($100/\sqrt{M}$ or its root mean square value for the series in question). Hence, such favourable results cannot be expected regularly in a long series of tests. However, the frequent occurrence of such good uniformity in our tests suggests that the errors due to our sampling technique, and those due to imperfect mixing of our aerosols, do not exceed the errors of random sampling for counts not exceeding 1500 per sample (coefficient of variation = $\pm 2·6$ %). In the air of crowded rooms much greater variations are found, due to irregular movements of air and persons.

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Table 1. *Uniformity of sampling*

Test		Decay rate of *Strep. salivarius* aerosol in spray room						Observed coefficient of variation	Calculated random error; root mean square of $(100/\sqrt{M})$
Consecutive samples of ½ cu. ft. each									
1	Plate counts	1176	993	832	709	596	516	—	—
	% deviation from logarithmic line	+2·3	−1·9	0·0	−2·6	0·0	+2·6	1·9	3·6
2	Plate counts	1332	1110	905	696	598	517	—	—
	% deviation from logarithmic line	−0·2	+1·2	+0·5	−5·7	−0·7	+5·0	3·1	3·5
Consecutive samples (10 cu. ft.) in quiet hospital wards—1 : 500,000 gentian violet blood agar									
3	Total organisms	84	84	98	85	—	—	6·7	11
	β-streptococci	24	22	24	23	—	—	4·0	21
4	Total organisms	34	26	30	29	31	25	10	19
	β-streptococci	9	9	10	8	6	10	16	33

Collection efficiency

An extensive series of tests was made with two similar slit samplers in tandem (method (1) above) in order to determine their collection efficiency under various conditions.

(*a*) *Effect of particle size, and of slit-plate distance.* This was investigated by using aerosols of different particle size varying from the finest sprays of separate cocci in distilled water to the coarsest spray we could obtain. Samples were taken from each type of aerosol at a constant rate of 1 cu. ft./min. at varying slit-plate distances, and the collection efficiency plotted against slit-plate distance (Fig. 3).

The curves fall into three sharply defined groups which correspond to the type of spray used, that is, to particle size. In the first group from the fine spray the three lowest curves show the collection efficiency for the finest particles—single (or diploid) organisms sprayed from distilled water. The efficiency for the 'A' coccus is slightly greater than that for *Strep. salivarius* or *Staph. albus*, as is to be expected from its greater size, but the differences between the curves, except in their central portions, are small. The steep fall in efficiency which follows an increase in the slit-plate distance from 2 to 10 mm. is very marked. The fourth curve of this group is that of *Strep. salivarius* sprayed from serum broth, and shows the influence of the evaporated solids from the broth in increasing the size of the bacteria-carrying particles. With this increase in particle size there is a moderate increase in efficiency, particularly with the greater slit-plate distances.

The second group consists of organisms sprayed from the medium spray. These show much higher efficiencies at slit-plate distances of 10–30 mm., corresponding to the larger size of the particles. The shape of the S-curve with *Strep. salivarius* is possibly due to a variation of particle size, the aerosol being composed of a mixture of single, diploid and chain forms. The last group, with the two highest curves, shows the efficiency of the samplers for

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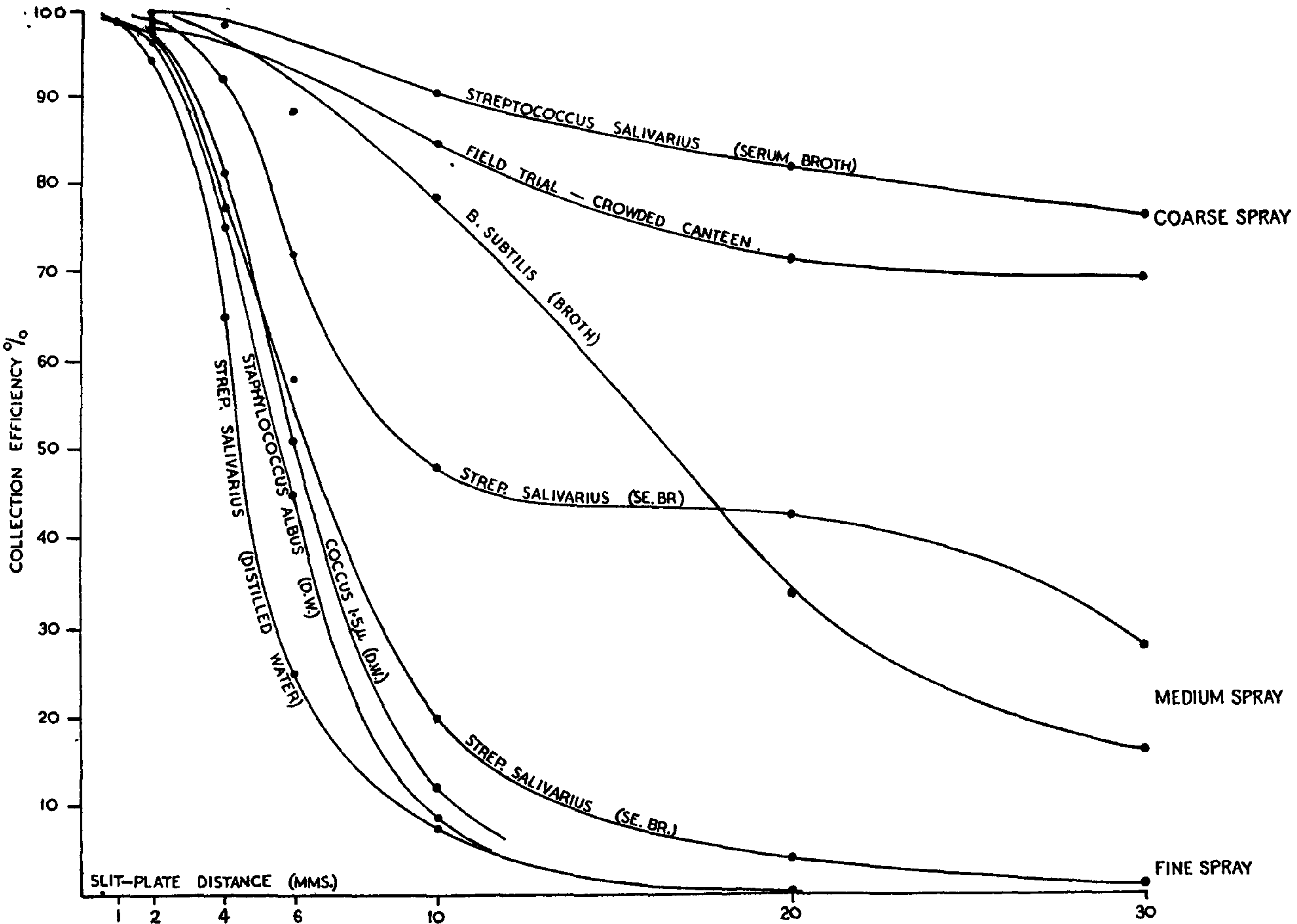

Fig. 3. Collection efficiencies of the slit sampler at various slit-plate distances. [The curve for *B. subtilis* is based on only one series of tests and is drawn to pass between the points at 4 mm. and 6 mm., which may be rather inaccurate.]

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the coarsest particles. The *Strep. salivarius* curve was obtained by using a coarse nasal spray giving large droplets, and the field test was from the air of a crowded military canteen. Calculations based on the sedimentation rate of canteen air particles, while admittedly uncertain, give an estimate of their size as about 10μ diameter. The relatively high efficiency of 70% at 20 mm. slit distance is striking confirmation of the large average size of these particles. The data from which these curves are constructed are given in Table 2, for the convenience of those who wish to calculate the errors to be expected when working at slit-plate distances greater than 2 mm. From 2 to 6 mm. the curves are not far from linear, so that the effect of small variations can easily be estimated.

Table 2. *Collection efficiencies at 1 cu. ft./min.*

		Slit-plate distance in mm.						
Organism	Conditions of spraying	1	2	4	6	10	20	30
Strep. salivarius	Fine spray in distilled water	99	94	65	25	7·5	<1	<1
Staph. albus	,, ,, ,,	98·5	96	74	45	8	—	—
Coccus A (1·5μ)	,, ,, ,,	98·5	97	81	51	12	—	—
Strep. salivarius	Fine spray in serum broth	—	97	77	58	20	4	1·6
Strep. salivarius	Medium spray in serum broth	—	99	92	72	48	43	28
B. subtilis	,, ,, ,,	—	98	98	88	78	34	16
Strep. salivarius	Coarse spray in serum broth	—	99·6	—	—	90	82	76
Mixed	Air of military canteen	—	97·5	—	—	84·5	71·5	69·5

(*b*) *Effect of rate of flow of air, i.e. of manometer pressure.* Owens showed that the deposition of very small dust particles became much more effective at pressures of 7–15 ft. of water across the slit than at low pressures. Although such pressures may be desirable for virus particles, we have concerned ourselves with nothing smaller than separate washed staphylococci and streptococci, and for these have found it simpler and safer to work at the suction head of 11 in. of water. The collection efficiency curves for various conditions at different rates of flow are shown in Fig. 4. The big fall in efficiency for the 2 mm. slit-agar distance at rates below 0·5 cu. ft./min. makes the slit sampler unsuitable for volumes of air less than 0·25 cu. ft., unless the length of the slit is cut down to one-half or one-quarter (with corresponding reduction in the area of Petri dish utilized). The fine spray was used for these tests.

If it is necessary to sample smaller volumes of air, a single jet $\frac{1}{16}$ in. or 1 mm. in diameter can be used instead of the slit.

When sampling very pure atmospheres there is, however, no harm in sucking at 2 cu. ft./min., if the pump is capable of it.

(*c*) *Effect of humidity.* Our efficiency curves have been measured at temperatures of 15–25° C. and relative humidities of from 30 to 70%. No difference was detected within this range. Owens found the use of a humidifier increased the efficiency of collection of small particles, since in moist air the fall of temperature caused by adiabatic expansion across his slit (made of thin microscope cover glasses) caused condensation of water droplets which facilitated deposition of the particles. We have avoided the use of a humidifier

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as troublesome for large volumes of air and difficult to sterilize, and by the use of a thick metal slit we have obtained a more robust instrument which seems quite efficient for bacteria. Thermocouple measurements show only a trifling fall of temperature across our slit, owing probably to the fact that it is

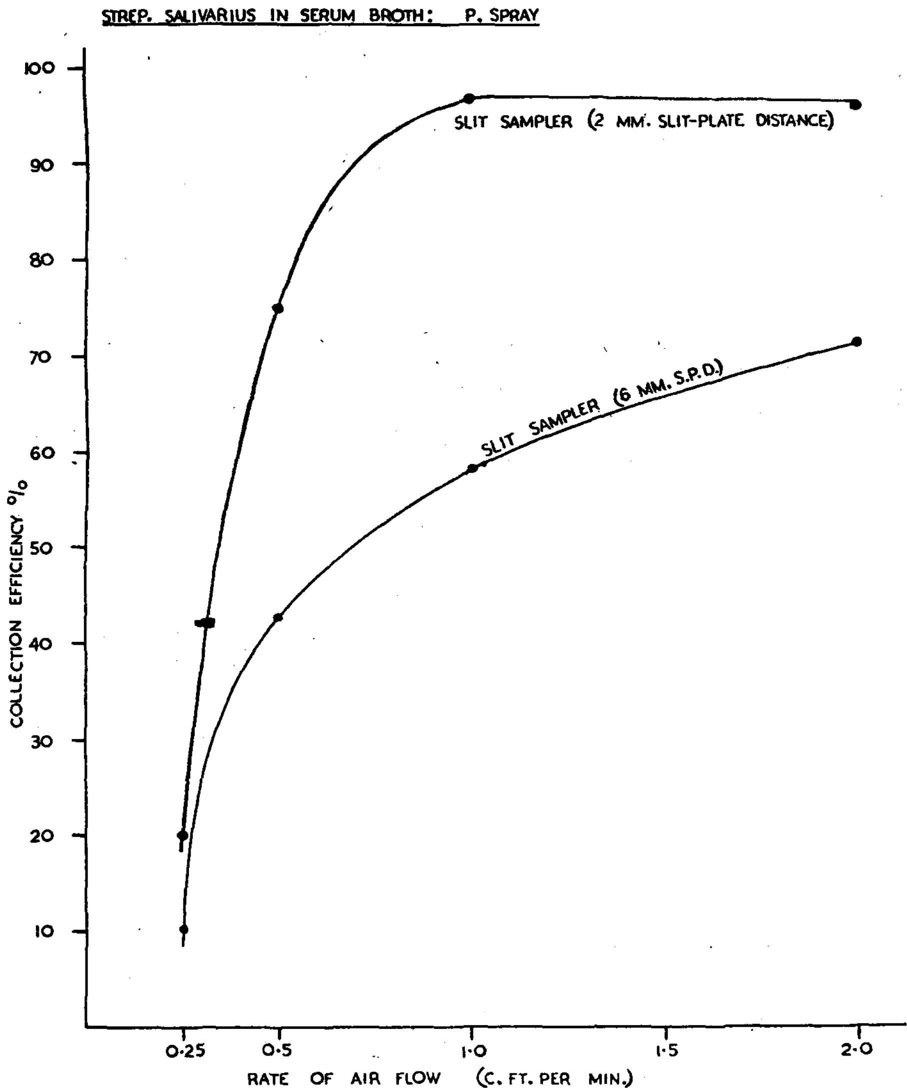

Fig. 4. Collection efficiencies of the slit sampler, etc. at various rates of air flow.

a metal tunnel 3 mm. long, i.e. 12 times as long as it is wide. Hence the air leaves the slit at approximately the temperature of the whole instrument. This keeps within half a degree of the room temperature.

(*d*) *Effect of size and shape of slit.* We have not studied many variations in size or shape, but have found that under our conditions of working a deep

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slit between jaws 3 mm. thick is rather more efficient than a shallow one, presumably owing to the production of a less turbulent air flow. It is important to avoid the use of jaws ground at 45° since under certain conditions these can cause serious loss of efficiency.

Test with a second type of sampler to determine numbers before and after passing the slit sampler (method (3) above)

Dr W. J. Elford kindly tested one of our slit samplers using an entirely different technique (to be published later) involving shaking with broth. On a fine aerosol consisting almost entirely of single cocci of *Staph. albus* in distilled water, he found the sampler to have a collection efficiency of 88%. As his apparatus would not allow of a rate of air flow greater than 8 or 9 l./min. the sampler used in this test was fitted with a slit only 6·3 mm. long instead of 27·5 mm. When tested by ourselves with the tandem method (1) this shortened slit gave an efficiency of 91% for a similar aerosol. This is in good agreement with the 88% found independently, although both are appreciably lower than our values for the normal slit.

6. Direct comparison with other methods

The Wells's centrifuge

This instrument has many advantages over other methods of sampling air for bacteria. It is quick to use, samples known volumes of air, and is free from many of the dangers of killing or inhibiting bacteria that occur in filtration methods. On the other hand, it is difficult to construct and costly to buy. There is often trouble due to spreading colonies, and counting may be difficult or impossible near the bottom of the tubes, where an appreciable percentage of the bacteria collect.

We were unable to test its collection efficiency by the tandem method owing to the difficulty of coupling a second instrument in series with the Wells's without disturbing the air flow. We therefore compared it with the slit sampler by taking simultaneous samples with the two machines from adjacent portions of air with such precautions as were possible to ensure uniformity of samples.

The Wells's tubes were lined with serum or blood agar, usually on the day before a trial. The tubes were left overnight, inclined mouth downwards, at 37° C. with cotton wool plugs so as to facilitate some draining. This left the medium dry enough to avoid streaks from liquid films, but always wet enough to form a small pool of liquid at the bottom during the incubation period after sampling.

The results of these tests are shown in Table 3, where the figures given represent the counts for the Wells's tubes expressed as percentages of the counts obtained from the slit sampler for equal volumes of air.

With fine, single-organism aerosols we were able to collect, with the Wells's centrifuge, anything from one-twentieth to one-half of the organisms

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collected by a slit sampler. The smallest bacteria-carrying particles sprayed from distilled water gave a collection efficiency of 5 or 6 %; when sprayed from serum broth the efficiency rose to 35–45 %. We could find no increase in efficiency with higher rates of flow than 1 cu. ft./min. The efficiency rose sharply with the coarser bacteria-carrying particles found in crowded rooms, where the Wells's counts were roughly three-quarters of those given by the slit sampler.

Some of these results agree well with those of certain other workers.

Thus, Macdonald (1940) reported an average collection efficiency for the Wells's centrifuge of 75 % for normal air-borne organisms, which agrees very closely with our mean figure of 74 %. He quotes, however, 98 % for sprayed *Chrom. prodigiosum*, but gives no data as to the size of droplets used. It seems probable that these were coarse.

Table 3. *Comparison of slit sampler and a Wells's centrifuge at different rates of air flow*

Percentage collection efficiency of Wells's air centrifuge compared with slit sampler taken as = 100

Organism	Type of bacteria-carrying particle	Site	Rate of flow cu. ft./min. 1·0	1·5	2·0
Strep. salivarius	Fine spray from distilled water	Spray room	5	—	—
Staph. albus	" "	"	6	—	—
Strep. salivarius	Fine spray from serum broth	"	36	31	36
Chrom. prodigiosum	" "	"	45	—	—
Air	Mixed dust, etc.	Air-raid shelter	76	—	—
Air	"	Canteen	72	—	—

Phelps (1940), using, we understand, a broth culture sprayed to form an aerosol of bacterial particles with a settling rate of 16 in./hr. (Wells, 1940), found the Wells's centrifuge to recover from one-third to one-half of the organisms which could be recovered by quiescent settling upon Petri dishes in 7½ hr. This appears in good agreement with our figures of 36 and 45 % for finely sprayed broth cultures. A settling rate of 16 in./hr. in still air would, for spheres of unit density, suggest a diameter of 2μ, which fits well with the position of our curve for the fine spray of *Strep. salivarius* in broth (Fig. 3). Hence it seems probable that the agreement with Phelps's figures is significant, although Phelps's data as yet published are insufficient to make comparisons reliable.

Our figures of 5 and 6 % for the Wells's centrifuge on fine sprays of *washed* bacteria are surprisingly low. It should, however, be stated that these particles are smaller and cleaner than those which appear to be most important in the transmission of bacterial air-borne disease.

We are indebted to the Hanovia Company of Slough, England, for the loan of the Wells's centrifuge used in these tests, and are glad to express our thanks to them for this.

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The funnel method

Hollaender & Dalla Valle described this method as collecting approximately 80% of spores of *B. subtilis* of about 2μ diameter, sprayed from a paint gun into a large chamber and allowed to settle for about 30 min. before sampling. We have found the method very easy to use, and the instruments simple to construct (given a suitable pump). In our hands the efficiency depended to an appreciable extent on the horizontal clearance (i.e. difference in diameter between the funnel and Petri dish) and hence required care in the selection of dishes of uniform size. We obtained the following relative counts by the funnel method, taking parallel samples with the 2 mm. slit sampler as 100: crowded room about 75; coarse spray of *Strep. salivarius* in broth 45; medium spray of the same 7; fine spray of *Strep. salivarius* in water 1½, and of *Staph. albus* in water 1.

We conclude that the funnel method can be useful for large particles as found in crowded rooms, but is not efficient enough for reliable work on the smaller bacteria-carrying particles.

The slit sampler may be regarded as an adaptation of the funnel method, arranged to work at much higher linear air velocities, and hence securing much higher efficiencies for small particles.

McConnell and Thomas's aeroscope

Tests of the filtration efficiency of this sampler were made by using it in tandem, with a 2 mm. slit sampler as the second instrument (method (2) above). These tests gave collection efficiencies ranging from 60 to 90% when using a fine spray of *Staph. albus* and *Strep. salivarius* in distilled water. However, tests of relative overall efficiency (method (4)) gave a value of only 18% for canteen air, and when using *Strep. salivarius* (fine spray in broth) we failed to grow any of the retained organisms. This appeared to be due, either to the difficulty of washing the organisms from the silk, or to the death of the organisms while in the water used in the aeroscope.

Sedimentation on open Petri dishes with agar jelly

It was recognized by Wells & Riley (1937) and by others that open dishes form an undesirably selective method for studying air-borne bacteria, since the larger particles alone sink in air at an appreciable rate. The open dish is not usually regarded as giving information on the number of bacteria in any given volume of air (see, however, Wells *et al.* 1940) but only as giving relative numbers suitable for comparative tests. We have, therefore, expressed our figures in terms of the ratio of bacteria found by the slit sampler in 1 min. (i.e. in 1 cu. ft.) to those found in a 3½ in. Petri dish for each minute of exposure. This ratio 'slit sampler/Petri dish' ranges from 5·5/1 for the air in a crowded canteen (for total organisms 5·5/1, for β-haemolytic streptococci 11/1), to 300/1–500/1 for *Staph. albus* or *Strep. salivarius* sprayed in broth from a fine

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spray, and from 1000/1 to 5000/1 if sprayed from distilled water. The smallest particles seem to deposit very irregularly, and the numbers found in consecutive plates may vary by 100 % or even more. We find roughly the same ratio in our spray room of 3000 cu. ft., whether the air is still or in moderate movement from a 12 in. electric fan (not blowing towards the plates).

This range of over 200 to 1 on the slit/Petri ratio is very striking proof of the selective action of open dishes when used for collecting bacteria. It is probably safe to say that such dishes are at least 200 times as effective for collecting large bacteria-carrying particles as for single washed air-borne bacteria. Hence great caution is needed in drawing inferences from tests made with this method.

The results obtained by sampling in a military canteen with slit sampler and with Petri dishes throughout the day are shown in Fig. 5. The Petri plates were exposed for 20 or 30 min. each, but their colony numbers have been divided by 2 or 3 to give the numbers collected in each 10 min. period, corresponding to the 10 min. collections with the slit sampler. This test was done with an old model of slit sampler having a distance from slit to agar of 20 mm. We now know that a 2 mm. distance would have given figures about 20 % higher.

The chief differences between the two methods shown in this test are (1) the slit sampler was much more effective in detecting β-haemolytic streptococci, which were only present in small numbers (about 0·1 per cu. ft. for most of the day); (2) the slit sampler was much more effective in detecting short-period changes in air content than the Petri dishes, owing to the shorter period in which it collected a number of organisms sufficient to constitute a satisfactory sample (i.e. one free from excessive random error).

This is well seen in the afternoon, for the period when the numbers of men present rose suddenly to 160, as well as for the lunch-time sweeping period.

In both samplers the medium used was blood agar containing 1/500,000 gentian violet. This dye checked the growth of moulds and many other saprophytes, and so increased the proportion of organisms showing α-haemolysis. By rendering it possible to take samples of 10 cu. ft. on one plate without overcrowding, the dye also facilitated the detection of the few β-streptococci present. Of the organisms showing α-haemolysis, which some workers (Buchbinder *et al.* 1938; Wells & Wells, 1936) use as an index of aerial contamination, a large number were staphylococci. The α-streptococci were not counted separately.

It is noteworthy that both the slit sampler/Petri dish ratio and the collection-efficiency curve for varying slit-plate distance indicate that the infected particles in canteen air are of an average size much larger than 1μ.

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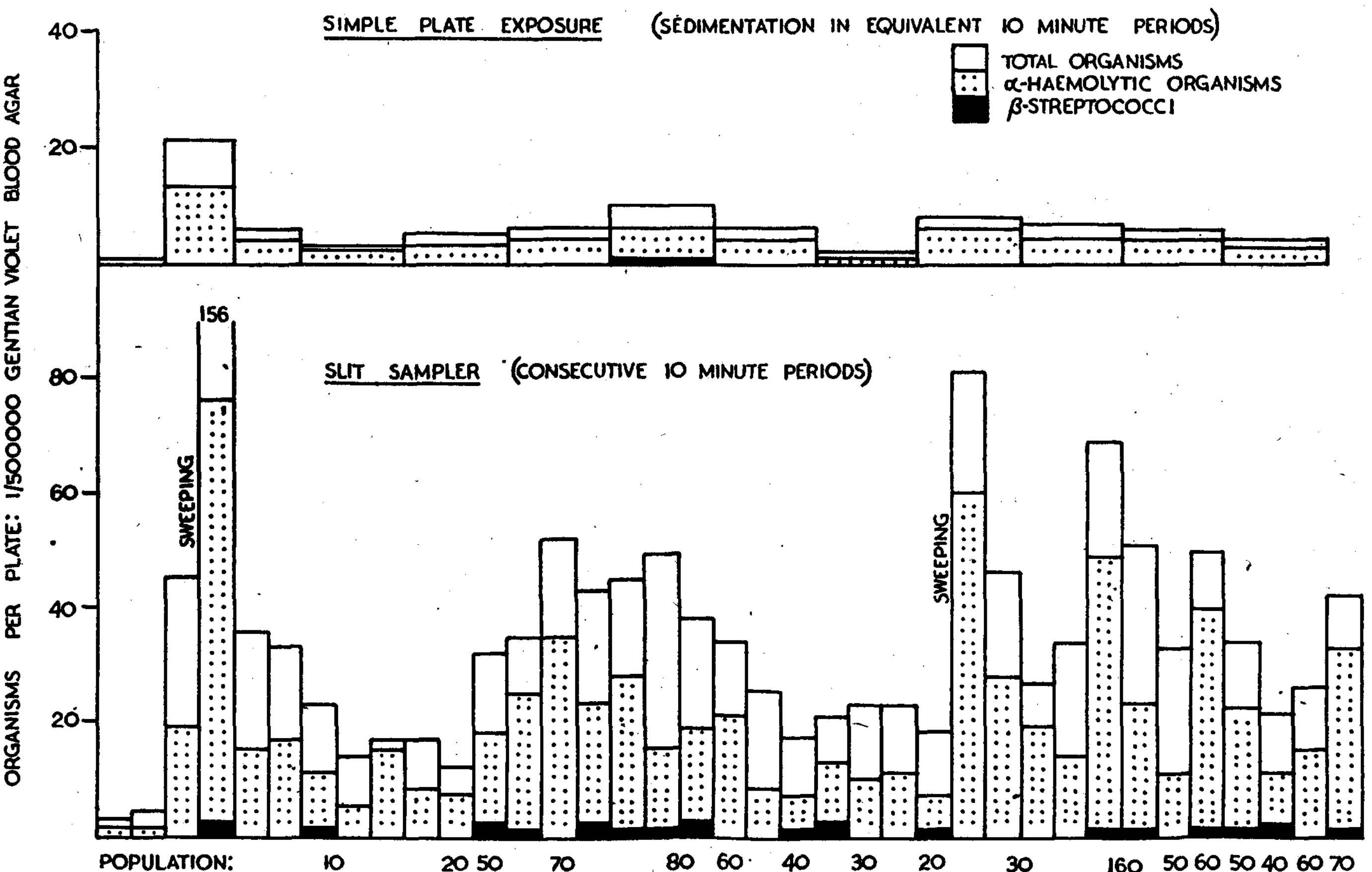

Fig. 5. Analysis of canteen air for a 6 hr. period. Upper section by Petri plates. Lower section by slit sampler.

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7. TESTS FOR POSSIBLE SOURCES OF INEFFICIENCY

Certain special sources of error which might be expected with the slit sampler have been examined as follows:

(1) *Death from concussion*

McConnell & Thomas have suggested that their failure to collect bacteria in a Greenburg impinger was due to the high internal stresses set up in rapidly moving bacteria when impinging on a solid copper surface. These stresses will be much reduced when impinging on to a soft agar surface as in our technique, and we think that the following tests show the killing effect to be negligible.

(*a*) *Effect of air flow on collection efficiency.* It was shown in the tests illustrated in Fig. 4 that the collection efficiency, that is $\alpha+\beta$ (p. 206), was roughly constant and close to 1·0 for rates of flow from 1 to 2 cu. ft./min. This is only possible if either (1) α and β vary in inverse sense over this range, or (2) neither α nor β varies appreciably, or (3) if β (or α) varies appreciably but is only a small fraction of 1·0. The first possibility can be excluded by the results of a series of tests which showed that the estimated number of organisms in a given aerosol was independent (when corrected for collection efficiency) of the rate of flow of air during sampling over the range 0·5 to 2·0 cu. ft./min. The second possibility is improbable unless that portion of β which is due to concussion is small, since if killing from concussion was appreciable at 1 cu. ft./min., the fraction left unkilled would be likely to diminish noticeably at double this velocity. Hence it is probable that if any organisms are killed by concussion their number is small.

(*b*) *Effect of collecting on to a bare glass dish.* In one of these tests *Staph. albus* sprayed from distilled water was collected in a slit sampler containing an empty glass Petri dish placed 2 mm. below the slit. This plate was later poured with agar at 44° C. The air leaving the first sampler was passed through a second sampler containing a normal serum-agar plate. The first (glass) surface retained only from 3·5 to 9% of the cultivable organisms, while the second (agar) surface grew a large fraction (average 92% in four different tests) of the organisms to be expected if none had been killed by hitting the glass. Similar tests on canteen air gave about 25% of the organisms on the glass surface and about 75% on the agar surface with no certain evidence of killing on the glass plate. These tests are rather crude, but it seems safe to conclude that the percentage of organisms killed on the glass surface was not much higher than that killed on an agar surface. This is only probable if the percentage killed by impact on an agar surface is very small. The impact velocity at 1 cu. ft./min. in our sampler is only about 0·6 of that in the Greenburg impinger.

It is interesting to note that the glass plate is a poor collecting surface under our conditions, and that the high efficiencies obtained with agar are

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due to certain favourable properties such as the softness and wetness of its surface.

(2) *Overdrying on prolonged suction*

The portion of agar directly under the slit receives an air current moving at about 270 ft./sec. This must cause local drying, and it is evident that while rapid re-moistening will occur by local diffusion as the portion of the plate concerned moves away from under the slit, the whole plate must get dried as rotation continues. This point was tested by sucking sterile air (sterilized by passing through a heated iron tube and then cooling to room temperature which was at about 17° C. with relative humidity of 50 %) over a serum-agar plate with continuous rotation for 1 hr. in the slit sampler. Two and a half minutes after this flow of air was stopped, the same sampler and plate were used for an aerosol of *Strep. salivarius* finely sprayed from broth culture. Directly after this a similar sample was taken on a normal serum-agar plate. The two plates gave counts identical within the error of test, although sufficient drying of the 1 hr. plate had occurred to lower the agar surface perceptibly in all parts except the centre, which did not come directly under the slit. On the other hand, a similar test continued for 2 hr. showed a serious loss of growing power, which was complete towards the inner end of the slit where the linear rate of rotation is lowest.

We conclude that in normal climates suction for 1 hr. is probably safe, but that in dry tropical climates, such long exposures should be given only after control test. It is, of course, only in very clean air that it would ever seem desirable to sample such large volumes, and in such cases there is rarely any objection to changing the plate at half-hour intervals.

(3) *Adhesion of organisms to entry tube or walls of slit*

Such adhesion is most likely to be serious with large particles such as are found in dusty air. A test in a crowded military canteen, with an initially sterile slit tube and slit, and 30 cu. ft. of air sucked in 30 min., showed bacteria collected on the plates equivalent to 1428 in the 30 min. period. At the end of the test the slit tube and slit were washed vigorously with broth, and from the washings thirteen colonies were obtained on plating out the whole amount. It appears that in canteen air less than 1 % of the particles adhered to the slit or slit tube.

(4) *Length of incubation period*

In sampling aerosols made from a single species of organism it is easy both to choose suitable culture conditions, and to show by tests that the incubation period is sufficient to grow to visible size nearly all the colonies that can be grown. Thus, in our laboratory efficiency tests, we have usually only incubated for 18 hr. at 37° C., since tests showed that further extension of incubation only caused a small increase in colonies counted. In sampling the air of crowded rooms the problem is much more difficult, owing to the different conditions which prove optimal for pathogens and for saprophytes.

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A further danger arises from the known tendency of single bacteria to take considerably longer than clusters of bacteria to grow to a visible colony (Garrod, 1935). By counting plates from canteen air at intervals up to 72 hr. we have shown that there is a progressive, and often considerable, increase in the number of colonies growing in the later periods. Our results have been very variable and suggest a varying heterogeneity of the aerial flora on different days. In one such series of tests using a sampler with slit-plate distance of 20 mm. in tandem with a 2 mm. sampler behind, 1356 colonies grew on the front plates and 362 on the back in 17 hr. At 64 hr. the respective counts were 1719 and 475, the calculated efficiencies from these two sets of figures being 79 and 78·3 % respectively. In another series of tests there was a 15 % increase in the total count over the same incubation period, while a third series showed a 63 % increase. The whole question of optimum incubation time for aerial organisms requires further investigation. We have been satisfied with the shorter incubation period for our field trials, as although progressive incubation increases the absolute number of colonies, we have not found any significant alteration in the percentage collection efficiency.

8. ALTERNATIVE METHODS OF USING SLIT SAMPLER

(1) *Use for counting total bacteria in a sample.* When it is desired to count total organisms instead of bacteria-carrying particles, the solid medium used in the Petri dish can be replaced by any non-volatile liquid that is harmless to bacteria and is sufficiently viscous to avoid being scattered by the air stream. After sampling, the liquid is mixed with sterile broth, shaken as much as is considered necessary for breaking up clusters, and plated out. We have tested medicinal paraffin for this purpose and found it messy but efficient. Inactivated blood serum was also found efficient.

(2) *Use for the study of dust.* The slit sampler can also be used for microscopic study of the air-borne particles by sucking direct on to a glass microscope slide, with or without an adhesive film. If used at 11 in. water pressure the smaller particles will not be efficiently collected in this way, but as Pl. IV (*c*) shows it is suitable for collecting bacteria and for studying them when present in sufficient numbers to make recognition easy. The textile fibres and other large particles found in the air of crowded rooms are easy to study in this way.

9. DISCUSSION

In considering methods of sampling air three considerations are of chief importance, namely Estimation Efficiency, Collection Efficiency, and Convenience in Use.

With regard to estimation efficiency we are in no better position than other workers in this field. We know of no method which can safely be assumed to count every viable organism in a sample of air, and in the absence of such a method we can only judge estimation efficiency by comparison with other

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imperfect methods. Such comparative tests as we have done have in every case given results favourable to the slit sampler. In any case, estimation efficiency must depend greatly on the suitability of the culture medium used to the organisms concerned. With mixed organisms, as found in dusty air, the fraction β of organisms collected but not grown, on any one medium, is probably very considerable. This, however, may be of little significance for the study of air-borne bacterial disease, since it is easy to choose suitable media for the pathogens likely to occur in any given atmosphere. As the result of indirect evidence, which does not amount to proof, we believe that for the common pathogenic bacteria, and for bacteria sprayed in the laboratory, the slit sampler has a high estimation efficiency, possibly as high as 0·9 for vigorous organisms.

In discussing collection efficiency we are on surer ground. The tests quoted give us confidence that the collection efficiency of the slit sampler for bacteria-laden particles of all sizes is high, and is satisfactory for all ordinary test purposes.

The third criterion, namely convenience in use, is of dominant practical importance. After taking some 4000 samples with slit samplers in the last 9 months, we have realized fully the great advantages shared by those methods of sampling which collect the organisms direct on to a solid culture medium and avoid the labours and dangers of plating out from fluid suspensions. With fluid-suspension methods it is often impossible to carry out the later operations at the place of sampling, and yet this is most important if loss or multiplication of organisms is to be avoided. The former occurs very readily in distilled water or saline (Wright, 1941) while the latter is common in broth.

The slit sampler is not as easy to carry about as the Wells's centrifuge, owing to its need for a separate pump. We have found no difficulty, however, in using it in crowded air-raid shelters, canteens, etc., and when a suitable hand or foot pump is available the slit sampler can be used without electric current. Given a suitable pump, the slit sampler is much easier to construct than the Wells's centrifuge, and its Petri dishes are more convenient to prepare and to count accurately than the centrifuge tubes. This becomes important when dealing with large numbers of samples.

The range of concentrations which can be measured accurately with the slit sampler is from about 1 to 10,000 per cu. ft., unless moulds or mixed organisms of widely varying growth rates are to be counted. In that case 1000 or 2000 per cu. ft. is as high as is convenient unless special precautions are taken.

We wish to express our thanks to Drs C. H. Andrewes, W. J. Elford and M. van den Ende for the supply of numerous bacterial cultures and other help, to Major-General H. Marrian Perry, late Director of Pathology, A.M.S., and Col. E. B. Marsh for allowing one of us to take part in this work, and to Mr E. Pitte for making the numerous models of slit sampler used in this work.

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SUMMARY

1. A slit sampler for air-borne bacteria is described, in which air is sucked through a narrow slit on to the surface of agar in an ordinary Petri dish.

2. This method has proved very rapid and simple in use, and has appeared more efficient than other methods against which it has been tested.

3. The collection efficiency for an aerosol consisting of *Staph. albus*, sprayed from distilled water as single cocci, is about 96 %. The method thus collects the finest bacteria-carrying particles almost as efficiently as coarser ones.

4. In contrast to this, the exposure of plain open Petri dishes appears to collect the larger particles in the air of crowded rooms at least 200 times as efficiently as it does single washed bacteria. This highly selective effect renders it advisable to use great caution in evaluating tests made in plain open dishes.

5. The methods of determining sampling efficiency, and their errors are discussed in detail.

APPENDIX

1. CONSTRUCTIONAL DETAILS

Two models of slit sampler are described, one for simple hand rotation and the other driven mechanically. The body is identical in each. The dimensions given are those to which our machines are constructed; they are convenient, but with the exception of the slit itself, need not be followed exactly.

Body (Fig. 1).

Box (1). Sheet metal with sweated joints. Front plate brass $7 \times 4 \times \frac{1}{8}$ in. with centra hole $4\frac{1}{4} \times 2\frac{3}{4}$ in. Directly behind this is a plate of thin sheet brass or copper with central hole $4 \times 2\frac{1}{2}$ in. The two plates together form a frame with a recessed opening into which the glass window ($4\frac{1}{8} \times 2\frac{5}{8}$ in.) fits conveniently and is held by a broad strip of adhesive plaster. Top plate brass $5 \times 4\frac{1}{2} \times \frac{1}{8}$ in., with central hole for tube (11) and hole for tube (6) centred 2 in. from front of plate and 0·92 in. from centre of plate. This hole must be located within about $\frac{1}{16}$ in. if good distribution of colonies is to be obtained on Petri dishes whose size varies from $3\frac{1}{4}$ to $3\frac{7}{8}$ in., as is commonly found. Bottom plate brass $6\frac{1}{2} \times 4\frac{1}{2} \times \frac{1}{8}$ in. Sides $2\frac{1}{2}$ in. deep bent in a rectangular U-shape from $\frac{1}{16}$ in. sheet metal—each side 4 in. long.

Disk for carrying Petri dish (2). Brass circle $3\frac{3}{4}$ in. in diameter, $\frac{1}{16}$ in. thick surfaced with sheet rubber. Nut sweated centrally on lower surface with care to see that spindle is perpendicular to surface of disk.

Spindle for simple model (3). Steel rod $4\frac{1}{2} \times \frac{1}{4}$ in. screwed one end to fit nut on (2). If this screw thread is cut with hand tools and is slightly skew, this defect must be corrected by sweating the nut on to disk (2) with the spindle screwed into the nut and held exactly perpendicular to the disk.

Slit tube (4). $2\frac{1}{8}$ in. long and $1\frac{1}{4}$ in. internal diameter brass tube fitted with a $\frac{3}{4}$ in. collar of same tube as sleeve (6) and cut with projection to fit sleeve slot. The slit tube should fit its sleeve closely, but should be free enough to allow of easy removal (in use the tube is held in the sleeve by a short length of wide bore rubber tube (7); bicycle tyre tube is very suitable for this). The projection on the sleeve collar is arranged to locate the long axis of the slit radially from the centre of rotation.

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Slit (5). Jaws can be made of bronze, stainless steel or glass. It is possible to use razor blades, but these tend to warp and are not recommended. The edges of the slit should be ground at 90°, i.e. the slit should be a tunnel with perpendicular sides. Jaws of $\frac{1}{8}$ in. thickness appear rather more efficient than thinner ones. For adjustment bronze jaws can be soldered with little difficulty to tube 4 if one jaw is firmly soldered and then the other is located by a feeler gauge or some piece of metal a little less than $\frac{1}{100}$ in. thick pressed between the two jaws while the second one is fixed. Steel or glass jaws are more trouble and are best fixed to a brass plate inside the slit tube. The slit should be adjusted to $\frac{1}{100}$ in. width and $1\frac{1}{10}$ in. length as closely as possible, the ends being filled with small strips of metal or solder as required.

Outlets (6 and 7). Brass tubes, for suction $\frac{3}{8}$ or $\frac{1}{2}$ in. bore, and for manometer $\frac{1}{4}$ or $\frac{1}{8}$ in. bore sweated to one side of box.

Height indicator (8–11). $2\frac{3}{4}$ in. length of brass tubing ($\frac{3}{8}$ in. external: $\frac{1}{4}$ in. internal) sweated into central hole of top plate and projecting $\frac{1}{8}$–$\frac{1}{4}$ in. inside box; slotted at top in front for $1\frac{1}{4}$ in. Fitted with steel rod, screwed at lower end to take $\frac{1}{2}$ in. diameter $\times \frac{3}{16}$ in. circular brass plate (the fit should be as close as is compatible with free, vertical movement. The rod should be lubricated with vaseline to reduce friction and to make the joint as air-tight as possible.) Front pin at upper end with single horizontal mark on it. Millimetre scale at side of slot with central graduation in red; graduated so that the line on the pin corresponds to the red line on the scale when the lower face of the small brass plate is 2 mm. below the slit jaws.

A very simple height indicator can also be made from a steel rod with coned point fixed through the roof to project exactly 2 mm. below the slit. The Petri dish is raised until the image of this steel point as seen reflected in the agar almost meets the point itself. The method is, however, difficult to use in a bad light or when irregularities in the glass sides of the Petri dishes obscure the line of sight.

Spindle bearing (12). Plain brass tubing lubricated with grease or thick oil. If a good fit and about 2 in. long the air leakage will be unimportant.

Leaks. When completed it is important to test the apparatus for leaks at 11 in. water pressure. Any leaks below 300 c.c./min. can be safely neglected unless they are close to the slit jaws, where they may deflect the air drawn through the slit. Leaks between the jaw pieces and the plates to which the jaws are screwed must be avoided for this reason and for fear of contaminating the agar surface.

Stand for hand rotation (Fig. 2*a*).

Material: wood, ebonite or bakelite. Top and base $7\times7\times\frac{1}{2}$ in. Sides $8\times4\times\frac{3}{8}$ or $\frac{1}{2}$ in. Top fitted with two brass pins (23) on one side and a wing nut and bolt (23 A) on the other for fixing box to stand. A large central hole is cut in the top plate to allow removal of the box and spindle. Brass cross bar (29) fixed 3 in. above base; drilled with central $\frac{3}{8}$ in. hole to which is sweated a brass nut (27) taking a Whitworth screw bolt (or plain screwed rod with a nut sweated at its lower end), $3\times\frac{3}{8}$ in., 12 turns/in. Locking nut (28) fitted to bolt to prevent accidental alteration in height after adjustment. Top of bolt drilled and coned to take end of spindle. Rotation is effected by an arm or disk fitted to the spindle. We have used a 3 in. diameter bakelite disk held by a grub screw (25).

Stand for mechanical rotation (Fig. 2*b*).

Side pillars (21) *of ebonite or plugged brass tube* (22, 24). Top and bottom plates of $\frac{1}{2}$ in. bakelite or $\frac{3}{16}$ in. brass. Well-seasoned hard wood can be used.

Gear wheel. The speed of rotation of the shaft and plate can vary over wide limits without causing any error. We have used a 39-tooth gear wheel so that with a gramophone motor running at the normal speed of 78 rev./min. one revolution occurs in 30 sec. Any number of teeth between 20 and 60 would do nearly as well. We are indebted to Mr M. Gilbee for

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cutting the worms and gear wheels used in these samplers, and in particular for constructing the pumps used with them.

Height adjustment. This is similar to a turnbuckle. The knurled, brass nut (26) contains two, short, square-cut threads, 4 t.p.i., one right hand and one left hand, with the bands about $\frac{1}{8}$ in. square. The spindle is cut in two, the upper section drilled out and slotted and the lower section ground to fit into the upper and held in place by a locking pin (18) which prevents independent rotation of the two halves. Each section carries a brass nut (19) cut respectively with right- and left-hand threads to correspond to the outer nut. The lower section is held by the gear wheel, so that turning of the knurled brass nut either raises or lowers the upper section of the spindle. We are indebted to Dr E. Schuster for suggesting this locking device, and designing and constructing the first screw adjustment of this type and the first of our pumps.

Accessories (Pl. II).

Manometer. Any type of water manometer, preferably fitted with a trap or splash head to prevent liquid being sucked into the box; 15 in. column with one graduation at 11 in.

Pump. Must suck not less than $1\frac{1}{4}$ cu. ft./min. We use small brass pumps made in this laboratory with an eccentric rotor and sliding vanes driven at about 4000 rev./min. by a $\frac{1}{8}$ H.P. electric motor. The ordinary laboratory water pump and the smaller laboratory vacuum pumps are of too low capacity. The larger sizes of suction outfits used in some operating theatres are suitable. Hand or foot pumps to suck 1 cu. ft./min. at 11 in. water pressure are practicable but fatiguing.

Adjustable by-pass. A simple glass or metal T-piece, with a side arm fitted with a short length of rubber tubing and a clamp.

2. SAMPLERS IN TANDEM AND HETEROGENEOUS ATMOSPHERES

If N_r be the number of particles of kind r in the sample and E_r the collection efficiency of the instrument for this kind of particle, then, using the nomenclature of 4 (1), p. 205,

$$N = \Sigma N_r,$$

$$N_3 = \Sigma N_r . E_r = N_1 \frac{\alpha+\beta}{\alpha},$$

$$N_4 = \Sigma N_r . E_r(1-E_r) = N_2 \frac{\alpha+\beta}{\alpha},$$

and the quantity

$$\frac{N_3-N_4}{N_3} = \frac{N_1-N_2}{N_1} = E' = \frac{\Sigma N_r . E_r^2}{\Sigma N_r . E_r}.$$

This, the apparent collection efficiency, bears no relation to, but is greater than, the true collection efficiency

$$E = \frac{N_3}{N} = \frac{\Sigma N_r . E_r}{\Sigma N_r},$$

for

$$\frac{E'}{E} = \frac{\Sigma N_r . \Sigma N_r . E_r^2}{(\Sigma N_r . E_r)^2}$$

$$= \frac{\Sigma N_r^2 . E_r^2 + \Sigma N_{r_1} . N_{r_2} . E_{r_1}^2}{\Sigma N_r^2 . E_r^2 + 2\Sigma N_{r_1} . N_{r_2} . E_{r_1} . E_{r_2}}$$

$$= 1 + \frac{\Sigma N_{r_1} . N_{r_2} . \{E_{r_1}^2 + E_{r_2}^2 - 2E_{r_1} . E_{r_2}\}}{(\Sigma N_r . E_r)^2}$$

$$= 1 + \frac{\Sigma N_{r_1} . N_{r_2} . (E_{r_1} - E_{r_2})^2}{(\Sigma N_r . E_r)^2}$$

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And, since N_r and E_r are always positive quantities,

$$\frac{\Sigma N_{r_1}.N_{r_2}.(E_{r_1}-E_{r_2})^2}{(\Sigma N_r.E_r)^2}$$

is a positive quantity, i.e. $\frac{E'}{E}>1$ or $E'>E$.

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