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THE EFFICIENCY OF VARIOUS LIQUID IMPINGER SAMPLERS IN BACTERIAL AEROSOLS

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The liquid impinger was first described by Greenburg and Smith (1922) as a dust cloud sampler. More recently the device has come into wide use for bacterial aerosol sampling, as it is often very convenient for this purpose both in laboratory apparatus and in the field. Rosebury (1947) and Henderson (1952) describe the use of the " Porton " development of the original device. For estimating the number of viable organisms trapped in the impinger fluid they use the technique of Miles and Misra (1938) in which serial dilutions of the fluid are plated out for subsequent incubation and colony counting.

Valuable features of the impinger when sampling airborne organisms are: (1) It is compact and inexpensive. (2) The sample fluid can be plated out simultaneously on differing media thus ensuring optimum growth conditions for organisms of interest. (3) An extreme range of airborne concentration can be accommodated by the serial dilution technique. (4) Virus aerosols can be estimated effectively, provided that they do not contain a high proportion of very small, single virus particles which are difficult to impinge. (5) The particle retention efficiency is very high, effectively all particles down to about 0·5μ being trapped in the impinger fluid. (6) It gives a measure of the number of individual viable organisms in an aerosol, not the number of infective particles or clusters of organisms. (7) It acts as its own constant-flow metering device. (8) It is unaffected by repeated autoclaving.

Impingers similar to that shown in Fig. 1 were originally developed by Prof. J. H. Gaddum and have been in use in this establishment for many years. Fig. 1 shows the modern all-glass Porton type. Suction is applied to the small side arm and draws air in through the curving intake tube and down through the impinging jet. The jet is a short length of capillary tubing and acts as a critical flow orifice, *i.e.*, when the suction reaches about half an atmosphere the flow in the jet attains sonic velocity (Druett, 1955) and any subsequent increase in suction cannot increase the jet velocity further. Provided therefore that there is at least half an atmosphere of suction the impinger operates at constant flow and, once calibrated, does not require a flowmeter. The sonic velocity air jet strikes the flat base of the flask, throwing out its burden of particles which become suspended in the violently agitated circulating liquid. The apparatus shown in Fig. 1 has a jet diameter of 1·1 mm. giving a flow rate of 11 litres/min. and it holds 10 ml. of liquid in the flask, which has an internal diameter of 1¼ inches. The flask is made long in proportion to its width so that splashes of liquid cannot be entrained out of the suction tube (Rosebury used a conical flask which splashes less owing to the broader base, and which stands safely on the bench). The distance between the jet and flask base is 4 mm.

It will readily be seen that the impingement process is of great violence, for in a distance of about 1 cm. particles are accelerated up to sonic speed (about 760 m.p.h.) and are thrown against the hard glass base of the flask at approxi-

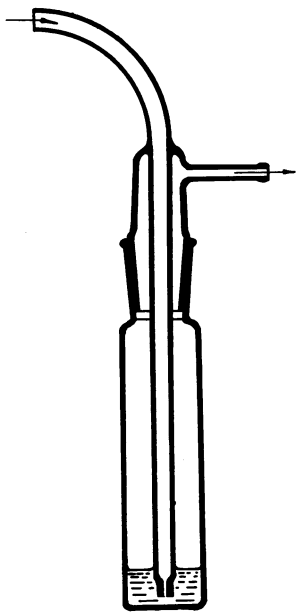

FIG. 1.—All-glass Porton impinger.

mately this speed. While this is known to shatter aggregated dust particles, experiments here with heat-resistant bacterial spores of *B. subtilis* had shown that their viability was in no way affected by the impingement process and it was assumed that this was so with more delicate vegetative cells. Doubt was thrown on this point by the advent of the American "Shipe" impinger (U.S. Chemical Corps, 1953) which in both countries was found to give consistently higher viable recoveries from various airborne vegetative cells than our Porton type of impinger. The Shipe impinger (Fig. 2A) has a critical orifice plate cemented into a side neck close to the base of the flask. The neck and air jet are inclined downwards and in the plan view are tangential to the side of the flask as shown. The air jet therefore strikes the base of the flask at a glancing angle and also imparts a very vigorous, non-splashing rotation to the impinger fluid. On the top of the Shipe flask fits a standard ground glass joint, with the suction tube blown into the upper portion.

There are three important differences between the Shipe and Porton impingers which might affect the viable recovery, as follows:—

(1) **Intake Differences.**—The particle-laden air stream reaches the impinging jet via a long curving tube in the Porton impinger whereas access to the Shipe jet is direct from the air. The curving tube traps by inertial effects the more massive airborne particles. This selective filtration was originally intended by Gaddum to simulate the human nose, though we now know that the existing tube is much less efficient than the nose. The standard suction rate of 11 litres/min. through the 8 mm. internal diameter intake tube of the Porton impinger gives a 50% retention in the bend of unit density particles of about 12μ diameter (the corresponding figure for the nasal system is about 4μ) while particles larger than about 18μ are entirely prevented from reaching the impinging jet. Hence the Shipe impinger will always show a higher recovery from aerosols containing large particles, the effect being emphasized by the number of organisms contained in a cluster being proportional to the cube of its diameter. An entirely separate effect is that the " intake efficiency", or ability of the larger particles to enter the Porton tube or the Shipe orifice, may be markedly different in the two impingers in cross draughts or winds, due to differences in the inflowing air streamlines. Clearly, any experiment designed to measure differences between these two, or any other impinging systems in respect of killing effects on viable particles, must be confined (*a*) to the use of particles small enough to be unaffected by tube losses or intake efficiency differences, or (*b*) to impingers with identical intake systems, or (*c*) to impingers fitted with a device such as the " pre-impinger " (May and Druett, 1953), which only permits particles of a small and known size to reach the backing impinger.

(2) **Jet Differences.**—The Porton impinger jet is formed from a short length of glass capillary whereas the Shipe jet is a hole drilled in a thin plate. The Porton jet, which is illustrated in Fig. 2B, can be criticized on two counts: (*a*) The transition from the 8 mm. intake tube to the 1 mm. jet is too abrupt, as shown by the fact that if the sample contains particles larger than about 3μ an appreciable deposition may be observed on the lead-in to the jet, even with a pre-impinger in use, and (*b*) there is no need for the jet to have any parallel portion at all for it to form a critical orifice. Davies, Aylward, and Leacey (1951) consider that a long, narrow, parallel-sided jet will, by shearing forces, break up particles of coal dust and it is possible that the shear forces in the capillary may in some cases have a bad effect on the viability of the more delicate organisms. Break up of cell clusters into their components is not in itself undesirable since the method of assay depends on each counted colony stemming from a single cell. Probably then the best shape for the Porton jet would be similar to that shown in Fig. 2C but leading down to a smaller hole with a very short parallel portion. The Porton jet was originally made in the Fig. 2B form because suitable capillary tubing can be readily obtained and quickly fused on the glass tube to give a standard flow-rate without further adjustment, and also probably because it was felt that a parallel portion was necessary to allow particles time to be accelerated by air drag up to the full jet velocity. The Fig. 2C form would be only slightly more difficult to make, should eliminate lead-in losses and would give satisfactory particle velocities because, as the results of this work will show, the full sonic velocity is not in general necessary to impinge the smallest bacterial particles. We shall, however, show that the Porton jet in its standard form will, with a suitable modification of the jet height, give similar viable recoveries to the Shipe, under the conditions of the tests.

(3) **Impingement Velocity Differences.**—After eliminating or making allowances for differences (1) and (2) above, the Shipe still showed an advantage in viable recovery. The remaining difference was therefore considered to be of fundamental importance. This is that the distance between the jet orifice and point of impingement (h in Fig. 2B) is 4 mm. for the Porton impinger with the air jet impinging vertically on the flat base and in the Shipe impinger is 30-40 mm. with oblique impingement

on the base. The violence of the impingement process is therefore much less in the Shipe, where the jet has time to lose much of its velocity by turbulent diffusion before striking the base.

In spite of the extreme violence of the impingement process in the Porton impinger, it is difficult to picture how an object with such a small mass as the bacterial cell (less than 10^{-12} g.) could be damaged by it. Conversion of the whole of the kinetic energy of the cell into heat could at the most only raise the temperature of the cell by a few degrees C., but owing to adiabatic cooling of the air jet and evaporative cooling of the impinger fluid in which the cell is immediately immersed, it seems doubtful if any actual temperature rise, however transient, could occur. It was thought that a permanently dry spot might exist in the centre of the impingement area or that cells might adhere firmly to the glass surface of the area and so escape suspension in the impinger fluid. In experiments to test these points these effects did not occur to any appreciable extent. Some form of mechanical trauma to the cell seems to be the only remaining possibility to account for loss of viability but we have been unable to devise any test for this point. A possible clue is that all the cells with which we have observed a killing effect are Gram-negative and these under manipulation are flabbier than Gram-positive cells (E. O. Powell, private communication). We have, however, not worked with Gram-positive cells other than the spores.

In view of the difference in performance of the two impingers we decided to carry out comparative tests of impingers with differing geometrical layouts and varying impingement velocity to see if any one form was superior in (*a*) the viable recovery obtainable from it and (*b*) the ease of modifying the Porton impinger to that form. We wished to retain unchanged the outer glassware of the Porton impinger because of the large stocks in hand together with matching ancillary equipment.

Types of Impingers Tested

The impinging systems tested are shown in Fig. 2. The Shipe impinger A and the standard Porton form B with jet clearance h = 4 mm. have already been described. The remaining types were all built into the standard Porton flask and all operate in the range 10·5-11·5 litres/min.

The " Sub-critical " Impinger, C.—This has subsonic jet velocity with the flow controlled by a critical orifice on the outlet tube of the impinger. For the tests, inlet tubes smoothly tapering down to jets of 2·2, 1·7, and 1·42 mm. were fitted, these being thought fine enough to give high impingement efficiency for 1 μ single cell particles. The clearance h was 4 mm. for each jet.

The " Venturi Jet " Impinger, D.—A jet of the form shown has the characteristic that constant flow starts at a much lower pressure drop than for the simple orifice or parallel-sided jet of Fig. 2A and B. Whereas the latter require at least half an atmosphere of suction for constant flow, the Venturi with the diverging cone at the optimum of 4° included angle requires only one-fifth of an atmosphere. Thus a considerable economy of suction power would be possible with this type of impinger.

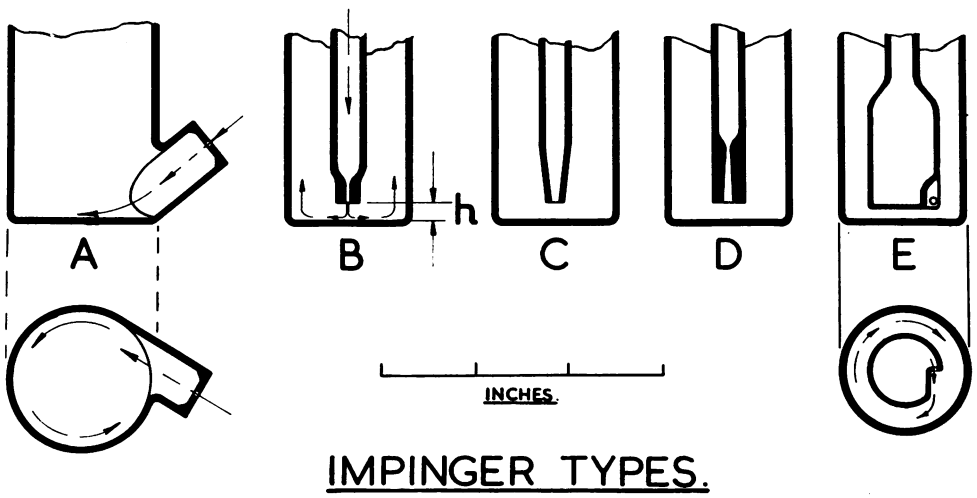

FIG. 2.—Impingement systems tested.

Venturi orifices are discussed by Druett (1955). The air velocity at the end of the expanding cone is, however, a function of the pressure drop, varying from low subsonic for small suction to supersonic when the suction approaches one atmosphere. The impinger would therefore have to operate at a specified pressure drop, otherwise the impingement performance would be expected to vary. The clearance h was 4 mm. The length of the diverging cone was 17 mm. and the lower orifice diameter was 2·3 mm. The throat diameter was 1·1 mm.

The "Swirling" Impinger, E.—This simulated the Shipe in that the impinging jet struck the lower wall of the flask tangentially and swirled the liquid round, but the swirl was more rapid than in the Shipe and the impingement distance shorter. The bottle-shaped end to the central tube seemed to be a suitable way of producing a jet at the required angle by boring through a kink in the wall, though there are of course other ways.

Bead Bubbler.—To confirm that impingement is the best means of sampling single-cell aerosols into liquids a bead-bubbler (not illustrated), as used for gas sampling, was tested. These bubblers comprise a glass tube of about 4 cm. internal diameter and 10 cm. long, half filled with glass beads of about 3 mm. diameter. The sample intake tube leads to the bottom of the bubbler with the outlet tube at the top and the indrawn air bubbles up through liquid filling the interstices of the beads. The 3 mm. beads were the finest that could be effectively used at our standard flow rate of 11 litres/min.

Procedure

(a) General.—Initial screening tests of the impingers described above were done in the single-cell aerosol generated by the Henderson spray apparatus (1952). The bacterial suspension sprayed was a mixture in known ratio of spores of *B. subtilis var. niger* and vegetative cells of *S. marcescens*. The robust *B. subtilis* spore was chosen as it is very suitable for obtaining the "filtration efficiency"* of an impingement system while the *S. marcescens* is delicate and usually gives a low viable recovery in the Porton impinger. Both organisms have the advantage of being non-pathogenic and can be separately cultured from samples of the same sampler fluid. They can readily be prepared as stable, non-clumping suspensions for spraying. By spraying and sampling the mixed suspension one can obtain strictly comparative figures for the filtration and viable recovery efficiencies in the same experiment. If *B. subtilis* spores are held in contact with nutrients from *S. marcescens* suspensions, either in liquid suspension or as an aerosol, they may undergo the first stages of germination, *i.e.*, lose heat resistance. In this state they are much more susceptible to damage in impingement processes. In the present experiments this was avoided by using *S. marcescens* suspensions almost free from nutrients with freshly heated spore suspensions to eliminate any heat-sensitive spores that may have been present and by strictly limiting the time of contact.

The screening tests with the single-cell aerosol having been completed, the type of impinger with most merit was selected and subjected to further comparative tests against the Porton impinger. These comprised tests in the open air in aerosols of coarser heterogeneous particles from the same mixed suspension as before and laboratory tests with pathogenic organisms.

(b) Bacteriological Preparation.—All *S. marcescens* suspensions were freshly prepared from the same strain. The *S. marcescens* was grown on peptone agar containing 1% mannitol, reaped into phosphate buffer, and used within 48 hours. This was done because the viable count of the suspension and its aerosol resistance fell off rapidly after this time. A single batch of *B. subtilis* spores was used throughout the tests. This had been prepared by growing on CCY agar, reaping in water, and heating at 60° C. to kill off vegetative forms. The mixed suspensions sprayed in the Henderson apparatus contained these organisms in approximately equal proportion, with a viable cell count of about 5×10^7 of each per millilitre of suspension. The actual counts were separately assessed before each experiment.

(c) Sampling and Assessment.—All aerosol samples were taken over a period of one minute in the laboratory work and were impinged into 10 ml. of phosphate buffer containing 0·25% "manucol" (sodium alginate). Dilutions were made in the same fluid. The function of the 'manucol" is to counter the effects of the so-called "agitation phenomenon" (Henderson, 1952). This is a progressive loss, which has been observed with spores, during agitation of the impinger fluid by the air jet. It is due to spores clumping together and sticking to the glassware. Manucol has been found to make this effect very small.

For assessment of *S. marcescens*, samples were plated on peptone agar containing 1% mannitol with the addition of 1/400,000 methyl violet to suppress spore growth. The *B. subtilis* was then assessed by heating the dilution tubes in a water-bath at 60° C. to kill the *S. marcescens* before plating on peptone agar.

In assessing any individual sample, colonies on quadruplicate culture plates were counted after suitable incubation. The plates assessed were those where the six uniform drops placed on them from the serial dilutions gave a colony count in the approximate range 100-200. Thus each sample was an estimate obtained from counting usually 400 to 800 colonies and was subject to an inherent standard error of roughly $\pm 5\%$ from the Poissonian distribution of samples of this size.

Each type of sampler was tested against a standard (h = 4 mm.) Porton impinger which took a simultaneous

* "Filtration efficiency" in this work is the ratio of the number of particles found in the sampling device to the total number of particles entering the sampler, the latter being estimated by the number on an efficient backing filter + the sample recovery. It is a purely physical concept.

reference sample from the same port via a Y-tube in the Henderson apparatus. Impingers were interchanged in successive comparisons. The viable recovery from the Porton impinger was taken as an arbitrary standard of unity in computing the relative performance of the other devices.

Notes on the Tabulated Results

(*a*) For convenience in presentation and comparison, the ratio of the number of viable cells of *B. subtilis* to the number of viable vegetative cells in the sprayed suspension, as estimated before each experiment, was numerically adjusted to unity. The same adjusting factor was then applied to the ratio found in the samples so that a direct comparison can be made between different tables.

(*b*) An " experiment " embraces work done in a single day with one setting of the spray apparatus and the same suspension throughout.

The difference which will be observed in aerosol concentration from experiment to experiment is mostly due to variations in the suspension mixtures made up before each.

A single " comparison " is derived from the two single, simultaneous samples taken by the Porton and test impingers. It was not possible always to make the same number of comparisons in each experiment owing to the considerable effort involved in the assessments and the pressure of other work.

(*c*) All " aerosol concentration " figures are the means of the estimations from the individual samples in the stated number of comparisons. They are expressed as viable cells ($\times 10^{-4}$) per litre of air drawn through the sampler. It is convenient to present the relative recoveries from different impingers in this way as their sampling flow rates usually differ a little.

The " ratios " are the means from the stated number of comparisons.

(*d*) To illustrate the reproducibility to be expected in this type of work, which involves several sources of error, standard deviations, $\sqrt{\Sigma(x-\bar{x})^2/(n-1)}$, are given with those aerosol concentrations or ratios which are of particular interest. Each standard deviation is derived from the spread of the values from each experiment, n being the number of comparisons shown in col. 2 in all tables except Table 9. The mean figures at the foot of the tables were obtained by giving equal weight to all individual comparisons summarized in the table.

Where only a small number of samples was involved or where the performance of the sampler under test was clearly poor, the standard deviations are not presented.

The average coefficient of variation from all experiments was 10·1%.

(*e*) In the last column of all tables except Tables 1 and 9 is given the ratio of the viable vegetative cell recovery from the test impinger to the viable *B. subtilis* recovery in the Porton impinger. We know that the loss of *B. subtilis* between spraying and assessment from the Porton impinger is very small (see below). In Tables 2, 3, 4(*c*), and 6 where we have efficient test impingers the ratio in the last column is close to unity and we can therefore make the important inference that no *S. marcescens* was killed in spraying or sampling. The tendency for the ratio to be rather greater than unity in Tables 2 and 3 will be discussed later. Where the ratio is significantly less than unity it indicates quantitatively the loss of *S. marcescens* due to the sampling process alone as, apart from the different test impingers, all experiments were carried out in an identical manner. The ratio can, however, only be taken as a measure of the " kill " of *S. marcescens* where the impingement velocity was high enough to give close to 100% filtration efficiency for the two different sizes of single cell particle, *S. marcescens* being smaller than *B. subtilis*. When the impingement velocity was low and the *S. marcescens*/*B. subtilis* ratio also low, this may be due to lower filtration efficiency for *S. marcescens* rather than loss of viability (an extreme case of this is seen in the bead-bubbler results, Table 7). The determination of filtration efficiencies for sensitive vegetative cells is very difficult and was not attempted.

Results with the Porton Impinger

***B. subtilis* Recovery.**—The basis of the tests was that the Porton impinger gives virtually 100% filtration and viable recovery efficiency with *B. subtilis* spores. This has been established by workers here and in America (Henderson, 1952; Rosebury, 1947), but a re-check was made at the start of the present series of tests. This was done by finding the number of viable *B. subtilis* cells recovered from the impinger and from a resin-wool filter sampling in parallel. The latter material is used in respirators, has an extremely high particle retention efficiency, and spores can readily be assessed from it by shaking with glass beads in buffer for subsequent plating out.

The results from six comparisons were: Aerosol concentration estimated by Porton impinger 5·33 ($\pm$0·34), by filter 5·32 ($\pm$0·23). These figures confirm the suitability of the Porton impinger as a standard for comparison. Further consolidation of this point has been obtained by experiments here with radioactively tagged cells. These experiments gave counts of cell material independent of viability by the purely physical Geiger count and showed that there was no loss of viability in filters or impingers when freshly heated spores had been used to produce single cell aerosols.

***S. marcescens* Recovery.**—The ratio of viable *S. marcescens*/*B. subtilis* recovered from the standard 1 : 1 mixed suspension (see Note (*a*) above) from nine experiments which gave 61 comparisons was 0·76±0·034. This indicates a loss of 24% of *S. marcescens* on sampling (Note (*e*)).

Effect of Modifications.—An obvious and very simple modification is to increase the distance h (Fig. 2) and allow the air jet time to lose velocity by turbulent diffusion before it strikes the base. This is permissible as the impingement velocity in the h = 4 mm. impinger is higher than it need be to trap all single cells, but we would expect a continuous fall in filtration efficiency as h is increased beyond a certain value.

To study the effect of increasing h, impingers with h = 18, 25, 30, 34, 40, 45, 50, 55, 60, 69, and 105 mm. were tested for single *B. subtilis* cell filtration efficiency and for viable *S. marcescens* recovery. All were filled with 10 ml. of collecting fluid. The results obtained are plotted in Fig. 3. The numbers by each plotted point are the numbers of comparisons made in obtaining that point. The upper curve of relative *S. marcescens* recovery shows a steady increase in recovery to a maximum of 134% as the distance h is increased to the optimum of 30 to 35 mm. At this distance the filtration efficiency as shown by the lower curve is only slightly lower than at h = 4 mm. Further increase in h gives the expected continuous fall in filtration efficiency which is reflected by the fall in the upper curve.

It was thought possible that the impinger with its jet raised to the optimum height of 30 mm. might show some variation in performance with pressure drop because of changes in expanding jet velocity or changes in splashing characteristics. To check this point a few tests were made and the results are presented in Table 1. Although a small change in filtration efficiency is indicated, there is no marked effect on the viable recovery figures even when the suction is only 8 in. of mercury, which is well below the critical flow value of 15 in. The low *B. subtilis* ratio in col. 9 at 15 in. Hg is thought to be experimental error, Table 2 giving more accurate figures for suction at this level.

Table 2 summarizes all the comparisons made at what Fig. 3 shows to be the optimum h value for this type of impinger. Column 8 shows that the modified form gave an *S. marcescens* recovery 34% greater than the Porton form. The values in col. 9 indicate that all the airborne *S. marcescens* was recovered in viable form from the modified impinger (see Note (*e*) above). Fourteen of the 20 comparisons summarized in col. 9 gave *S. marcescens*/*B. subtilis* ratios exceeding unity, however. This is statistically significant and implies that there was some small loss of *B. subtilis* in the Porton impinger, perhaps by "agitation" effects in spite of the "manucol",

TABLE 1

IMPINGER (*h* = 30 mm.) WORKING AT VARIOUS SUCTION LEVELS COMPARED WITH PORTON IMPINGER AT CONSTANT SUCTION

Negative Pressure on *h* = 30mm. Impinger (In. Hg.)	No. of Comparisons	% Filtration Efficiency		Aerosol Concentration of				Ratio (%) $\frac{h = 30 \text{ mm.}}{\text{Porton}}$ for	
				B. subtilis from		*S. marcescens* from			
		Porton Impinger	*h* = 30 mm. Impinger	Porton Impinger	*h* = 30mm. Impinger	Porton Impinger	*h* = 30mm. Impinger	*B. subtilis*	*S. marcescens*
8	2	99·9	94·0	10·06	9·77	7·96	10·00	97	126
15	2	99·9	97·5	9·61	9·06	6·84	9·00	94	132
27	2	99·9	99·7	8·96	8·65	5·99	7·81	97	130

TABLE 2

IMPINGER (*h* = 30 mm.) TESTED AGAINST PORTON IMPINGER

Experiment No.	No. of Comparisons	Aerosol Concentration of				Ratio (%) of Test/Porton Impingers for		Ratio (%) of *S. marcescens* from Test Impinger / *B. subtilis* from Porton Impinger (Col. 6/3)
		B. subtilis from		*S. marcescens* from				
		Porton Impinger	Test Impinger	Porton Impinger	Test Impinger	*B. subtilis* (Col. 4/3)	*S. marcescens* (Col. 6/5)	
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
1	6	8·53±0·45	8·27±0·56	6·90±0·94	8·78±1·55	98±11	129±34	105±21
2	6	5·27±0·26	5·42±0·33	4·49±0·38	5·89±0·34	103± 5	132±6	112±4
3	2	9·45	9·03	6·32	9·00	96	142	95
4	2	9·36	9·31	7·31	9·21	99	126	98
5	2	7·45	7·39	6·00	9·05	99	151	121
6	2	6·32	5·75	5·23	7·38	91	141	116
Means of	20	—	—	—	—	99± 8	134±19	108±14

Filtration efficiency of *h* = 30 mm. impinger = 97·4%±0·6 (8 tests).
Entries are, Sample mean ± estimated population standard deviation.

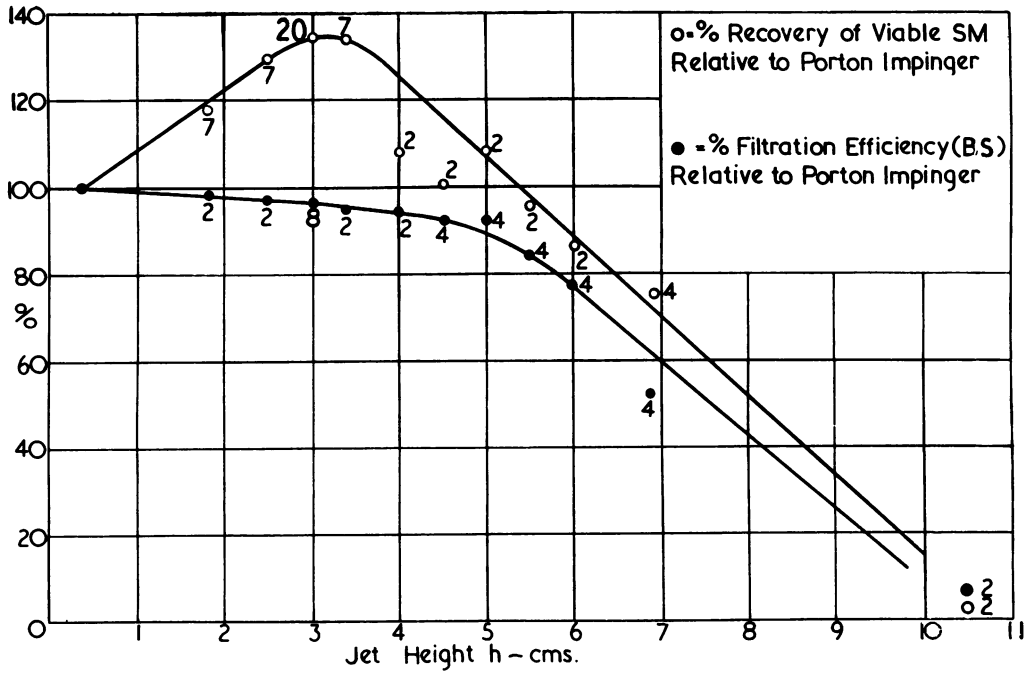

FIG. 3.—Effect of varying impingement distance in Porton impinger. The numerals by each plotted point give the number of tests made in establishing that point. BS = *B. subtilis*; SM = *S. marcescens*.

though this is contrary to the filtration efficiency results.

Results with the Shipe Impinger

Comparison of the means at the foot of Table 3 with those of Table 2 shows that the two types of impinger concerned gave effectively identical results. Their filtration efficiencies are also closely similar. The filtration efficiency and *S. marcescens* recovery figures relative to the Porton impinger also agree very well with those reported by American workers (U.S. Chemical Corps, 1953).

The figures in col. 9 are again very difficult to account for. Statistically the overall mean of 113% is significantly greater than unity as 12 out of the 15 comparisons yielded figures above unity.

Results with the Sub-critical Impinger

Of the three jet sizes tested it is clear from Table 4 that the two larger ones (*a*) and (*b*) do not compare with the Shipe or h = 30 mm. impingers in *S. marcescens* recovery (col. 8). This is because the relatively low jet velocity gives a low impingement efficiency.

The 1·42 mm. jet(*c*), however, gives a performance which is not significantly different from the mean

TABLE 3
SHIPE IMPINGER TESTED AGAINST PORTON IMPINGER

Experiment No. (1)	No. of Comparisons (2)	Aerosol Concentration of *B. subtilis* from Porton Impinger (3)	Aerosol Concentration of *B. subtilis* from Test Impinger (4)	Aerosol Concentration of *S. marcescens* from Porton Impinger (5)	Aerosol Concentration of *S. marcescens* from Test Impinger (6)	Ratio (%) of Test/Porton Impingers for *B. subtilis* (Col. 4/3) (7)	Ratio (%) of Test/Porton Impingers for *S. marcescens* (Col. 6/5) (8)	Ratio (%) of *S. marcescens* from Test Impinger / *B. subtilis* from Porton Impinger (Col. 6/3) (9)
1	6	6·27±0·31	6·27±0·17	5·35±0·46	7·26±0·53	100± 5	136±13	116
2	2	8·43	7·37	6·98	8·00	87	115	95
3	3	8·17	8·55	7·67	10·03	105	131	123
4	2	9·02	8·16	7·12	10·11	91	142	112
5	2	7·45	7·04	6·03	8·08	94	134	109
Means of 15	—	—	—	—	—	97±7	133±15	113±11

Filtration efficiency of Shipe impinger = 98·2%±0·8 (8 tests).

TABLE 4

SUB-CRITICAL IMPINGERS TESTED AGAINST PORTON IMPINGER

Experiment No. (1)	No. of Comparisons (2)	Aerosol Concentration of *B. subtilis* from Porton Impinger (3)	Aerosol Concentration of *B. subtilis* from Test Impinger (4)	Aerosol Concentration of *S. marcescens* from Porton Impinger (5)	Aerosol Concentration of *S. marcescens* from Test Impinger (6)	Ratio (%) of Test/Porton Impingers for *B. subtilis* (Col. 4/3) (7)	Ratio (%) of Test/Porton Impingers for *S. marcescens* (Col. 6/5) (8)	Ratio (%) of *S. marcescens* from Test Impinger / *B. subtilis* from Porton Impinger (Col. 6/3) (9)
(*a*)								
1	3	5·25	4·13	4·17	3·02	79	72	59
2	3	6·19	4·72	5·22	4·37	76	84	71
Means of 6						78	79	65
(*b*)								
1	3	5·07	5·29	3·84	4·33	104	113	86
2	3	6·20	5·50	5·82	5·82	89	100	94
3	3	5·39	5·25	4·44	4·69	96	106	87
4	3	6·18	5·48	5·58	5·71	89	102	93
Means of 12						96 ± 16	106 ± 15	90 ± 17
(*c*)								
1	4	8·93	9·09	6·99	9·00	102	130	101
2	4	10·86	11·43	8·53	11·01	103	129	101
Means of 8						103 ± 8	130 ± 14	101 ± 9

(*a*) Sub-critical jet 2·2 mm. dia. Filtration efficiency 72·5% ± 5·1 (6 tests) at 11 l/min.
(*b*) Sub-critical jet 1·7 mm. dia. Filtration efficiency 96·6% ± 0·7 (12 tests) at 11 l/min.
(*c*) Sub-critical jet 1·42 mm. dia. Filtration efficiency 99·80% ± 0·02 (4 tests) at 11 l/min.

figures of Table 2 and 3, cols. 7 and 8. The filtration efficiency is also very good. The velocity in the jet and 4 mm. below it at the point of impingement when running at 11 litres/min. would be a little below 300 m.p.h. which is similar to a value measured at the impingement point in the h = 30 mm. impinger. The pressure drop across the sub-critical jet at this flow rate is only about 1·2 in. Hg. The effect of the relatively low velocity and pressure drop as compared with the critical-flow impingers is that splashing, liquid loss through evaporation, and evaporative cooling are greatly reduced. In a critical flow impinger evaporation losses can become serious in sampling periods longer than one minute, especially at high temperatures and low humidities, and the liquid can freeze at low humidities and temperatures.

According to the work of Rang and Wong (1952) on circular impinging jets, the velocity given by this 1·42 mm. diam. jet at 11 litres/min. running dry is slightly greater than that required to give 100% impingement efficiency for 1 micron unit density spheres, and this jet was in fact chosen for that reason. The filtration efficiency figure, 99·8% for the single *B. subtilis* spores, is therefore as was hoped for this jet, without allowing for the enhancement of retention which the presence of the impinger fluid should give. The small quantity of spores which always penetrate through the impinger even with the most efficient jet are probably carried through by minute droplets originating in the violent splashing of the impinger fluid.

The filtration efficiency figures for the 1·7 mm. and 2·2 mm. sub-critical jets (96·6% and 72·5%) are higher than Rang and Wong's figures (90% and 25%) would suggest, but this can be attributed to the presence of the impinger fluid.

The mean value of col. 9 in Table 4(*c*) indicates complete recovery of all sprayed *S. marcescens* and there is no anomaly suggesting some *B. subtilis* loss as in Tables 2 and 3.

Results with the Venturi Jet Impinger

The performance of this type of jet at three suction levels, namely 6 in. Hg (the minimum suction for critical flow in this type of jet), 15 in. Hg, and 25 in. Hg, is shown in sections *a*, *b*, and *c* of Table 5. Col. 8 indicates that there is some improvement over the Porton type in *S. marcescens* recovery at the two lower suction levels, where the impingement velocities would be relatively low, but the diameter at the bottom of the nozzle, which is one of the parameters determining impingement efficiency, is in this case apparently too great for the efficiency to be very high at the velocity obtaining. The col. 9 figures are low because of the low filtration efficiency (c.f. Note (*e*) above). At the high suction level (Table 5(*c*)) the impingement velocity is probably in the supersonic region though this is not amenable to reliable calculation. The filtration efficiency is now very good but the *S. marcescens* recovery relative to the Porton impinger (col. 8) has dropped to a very low level which can only be due to the high impingement velocity. Col. 9 indicates that about half of the *S. marcescens* is " killed " at this high suction and velocity level.

TABLE 5

VENTURI JET IMPINGER TESTED AGAINST PORTON IMPINGER

Experiment No.	No. of Comparisons	Aerosol Concentration of *B. subtilis* from		Aerosol Concentration of *S. marcescens* from		Ratio (%) of Test/Porton Impingers for		Ratio (%) of *S. marcescens* from Test Impinger / *B. subtilis* from Porton Impinger (Col. 6/3)
		Porton Impinger	Test Impinger	Porton Impinger	Test Impinger	*B. subtilis* (Col. 4/3)	*S. marcescens* (Col. 6/5)	
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
(*a*) 1	10	8·82	8·05	6·70	7·25	91	108	82
(*b*) 1	10	8·93	8·37	6·83	8·12	94	119	91
(*c*) 1	3	7·06	6·94	6·05	4·65	98	77	66
2	4	6·50	5·85	5·65	3·55	90	63	55
3	4	8·64	8·24	6·71	3·60	95	54	42
Means of 11						94	64	54

(*a*) Venturi jet operating at 6 in. Hg pressure drop. Filtration efficiency 92·5% (7 tests).
(*b*) Venturi jet operating at 15 in. Hg pressure drop. Filtration efficiency 98·5% (7 tests).
(*c*) Venturi jet operating at 25 in. Hg pressure drop. Filtration efficiency 99·57%±0·11 (3 tests) (11·6 l/min.)

Table 5 shows clearly how the performance of this type of impinger varies markedly with the applied suction.

Results with the Swirling Impinger

The mean figures of Table 6 show a marked improvement over the Porton impinger, though not so great as in Tables 2, 3, and 4. When running, this impinger gives a very vigorous swirl with little splashing.

Results with the Bead Bubbler

The figures in Table 7 show that in spite of the thick bed of fine beads through which the sampled air bubbles, filtration efficiency with these small particles is low. No killing effect on the vegetative cells is indicated and for coarser aerosols the method would probably be entirely effective.

Review of Above Work

The tables show that the Shipe, h = 30 mm., and sub-critical (*c*) impingers give performance figures of high and equal merit. The Venturi jet and swirling types were inferior for reasons given above, and, although swirling types could no doubt be developed to give a very good performance, they would always be more difficult to make than the h = 30 mm. and sub-critical types.

The simplest modification of our existing large stocks was clearly to raise the jet of the Porton impinger to 30 mm. by shortening the inlet tube, although fundamentally the sub-critical type is

TABLE 6

SWIRLING IMPINGER TESTED AGAINST PORTON IMPINGER

Experiment No.	No. of Comparisons	Aerosol Concentration of *B. subtilis* from		Aerosol Concentration of *S. marcescens* from		Ratio (%) of Test/Porton Impingers for		Ratio (%) of *S. marcescens* from Test Impinger / *B. subtilis* from Porton Impinger (Col. 6/3)
		Porton Impinger	Test Impinger	Porton Impinger	Test Impinger	*B. subtilis* (Col. 4/3)	*S. marcescens* (Col. 6/5)	
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
1	1	9·61	10·49	6·49	9·52	109	147	99
2	2	8·29	8·40	6·78	10·10	101	149	120
3	2	7·45	6·90	6·03	7·53	93	125	101
4	3	7·13	6·93	5·54	6·76	95	122	95
5	3	8·19	8·21	6·80	7·00	100	103	85
6	3	6·80	6·54	5·63	6·68	96	119	98
Means of	14					99± 8	124±18	99±13

Filtration efficiency 99·6% (3 tests).

TABLE 7

BEAD BUBBLER TESTED AGAINST PORTON IMPINGER

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
1	1	8·92	4·54	7·00	4·45	51	64	50

Filtration efficiency 67·2% (1 test).

TABLE 8

RAISED (h = 30 mm.) IMPINGER COMPARED WITH PORTON IMPINGER FOR THE RECOVERY OF VARIOUS VEGETATIVE ORGANISMS

Organism	No. of Comparisons	Aerosol Concentration of *B. subtilis* from		Aerosol Concentration of Vegetative Organism from		Recovery Ratio (%) of Raised/Porton Impingers for		Ratio (%) of Vegetative Organism from Raised Impinger / *B. subtilis* from Porton Impinger
		Porton	Raised	Porton	Raised	*B. subtilis*	Vegetative Organism	
S. marcescens	20	data from Table 2				99± 8	134±19	108±14
P. tularensis	6	4·50	4·19	0·51	0·67	94±14	132±11	16± 3
P. pestis	6	5·50	5·29	5·39	5·40	96± 6	100± 9	98± 6
B. suis	6	4·59	4·35	4·73	4·76	96± 9	101± 7	104±13

perhaps more attractive with its constant small pressure drop, smaller liquid evaporation and cooling, reduced chance of jet blockage and with its smooth lead-in to the jet.

The decision was taken to concentrate on the h = 30 mm. type and it was then designated the " raised " impinger.

Further Tests on Raised Impinger

Table 8 shows results obtained with additional vegetative organisms in the Henderson single-cell aerosol apparatus. With *P. tularensis* the " raised " impinger shows an improvement over the Porton type similar to that for *S. marcescens* (col. 8). Col. 9 shows that the actual recovery of *P. tularensis* was only a small fraction of that sprayed but we had reason to believe that most of the loss took place during the spraying and drying of this extremely delicate organism.

With *P. pestis* and *Br. suis* the " raised " impinger gave the same high recovery as the Porton type but these organisms are known to be very robust in the conditions of the Henderson apparatus and there was no reason to expect loss of viability in the Porton impinger.

Open-air Work

To simulate naturally occurring aerosols more closely than did the laboratory work, tests were carried out over open ground using a small compressed air atomizer to produce an aerosol of heterogeneous particle size. The suspensions sprayed were of mixed *B. subtilis* and *S. marcescens*, similar to those used in the laboratory tests, except that 2% of glycerol was added in an attempt to protect the *S.marcescens* from death when the sprayed droplets dried down to equilibrium with the atmospheric humidity.

Sampling was carried out by small groups of impingers. Raised and Porton types were in close pairs so that as far as possible the two types received the same dose of aerosol. Each impinger was fitted with a pre-impinger (May and Druett, 1953) which allowed only the respirable fraction ($<5\mu$) of the sprayed aerosol particles to reach the impinger. The groups of impingers were placed down wind at distances which roughly depended on the prevailing wind speed, the object being to allow the droplets time to dry to equilibrium with the atmosphere. Air temperatures were in the 40°-55° F. range and humidities usually exceeded 80% R.H.

Results of the tests are summarized in Table 9. The reason for the great variability in total cell recovery from test to test is partly due to the variable distance down wind and partly to the chance as to whether or not the small group of impingers happened to stand in the region of peak concentration of the diffusing swathe of aerosol proceeding down wind from the spray. The striking feature of this series of tests is that the recovery ratios in the last

TABLE 9

RAISED IMPINGERS TESTED AGAINST PORTON IMPINGERS FOR RECOVERY OF *B. subtilis* AND *S. marcescens* FROM HETEROGENEOUS SPRAY IN OPEN AIR

Test No.	Total Cells in Pools ($\times 10^{-4}$) of *B. subtilis*		Total Cells in Pools ($\times 10^{-4}$) of *S. marcescens*		Ratio (%) of Raised/Porton for	
	Porton	Raised	Porton	Raised	*B. subtilis*	*S. marcescens*
1	640	765	247	828	120	335
2	960	1225	394	1100	128	279
3	28·4	48·0	14·0	44·7	169	319
4	39·6	56·4	23·0	67·5	143	293
5	55·5	57·8	29·3	75·4	104	258
6	58·5	53·8	17·6	24·8	92	141
7	64·0	61·6	42·9	65·4	96	153
8	58·3	64·6	14·2	52·6	110	372
9	456	372	103·5	256·5	81	248
Totals or means	2360	2704	886	2515	116±28	266±78

two columns show a much greater advantage to the " raised " impinger than did the laboratory work (Table 2). In the open air the " raised " impinger recovered nearly three times as much *S. marcescens* as the Porton impinger. The totals at the foot of cols. 2 to 5 indicate that the " raised " impinger is again recovering all the *S. marcescens* sprayed while the Porton impinger kills about two-thirds of this organism.

We attribute this marked difference between the laboratory and open-air work to the cells being in a more sensitive state when sampled in the open, where they have been subjected to the rigours of airborne suspension for a much longer period than in the Henderson apparatus and probably in less congenial conditions. They are therefore more readily killed by the violent treatment of the Porton impinger. The difference in particle size between the laboratory and open-air work (ca. 98% of single cells in the laboratory aerosol, clusters up to 5μ in the open-air aerosol) may also have some bearing on the difference in results, though if this is so the reason is obscure.

The ratios in the last column of Table 9 show rather more variation from test to test than did the laboratory work but this is to be expected because of the lack of control of variables in field work in general compared with laboratory work.

Discussion

The work shows that sonic-velocity impingement, as a means of sampling viable airborne bacteria, is too violent for the more sensitive types of cell. A system operating under liquid with a velocity at the point of impingement not exceeding about 300 m.p.h. is sufficient to trap virtually all individual airborne bacterial cells and no loss of viability can then be shown for the sensitive vegetative cells we have tested. *Serratia marcescens*, which was used in most of our tests, is one of the most sensitive of organisms to adverse treatment. There seems to be no reason why the best types of impinger selected by its use should not also be superior with other sensitive organisms. The reservation must be made that owing to the great inherent variability of living matter, our results will not necessarily be quantitatively reproducible with other strains or types of micro-organism. Also it is possible that cells which have been greatly weakened by prolonged exposure to the airborne state might be unable to withstand even very gentle impingement.

All our work was done with impingers operating at about 11 litres/min. While it seems reasonable that the results should apply to other flow rates, some work by Rosebury (1947) suggests that sonic velocity impingement at 2·5 1/min. gives superior viable recovery to similar impingement at 11 1/min.

Of the various types of impinger tested the sub-critical type seems to be of the highest general merit, for reasons already enumerated. A critical orifice can be incorporated into the outlet tube to make a constant flow system. Effectively all the viable cells which enter the jet of the sub-critical impinger may be recovered unchanged from the impinger fluid.

The so-called Shipe and " raised " impingers, which embody a sonic velocity critical jet with the point of impingement at about 3 cm. from the jet, give equally high performances and the most suitable one of the three types to use is largely a matter of choice. Other impinger types tested were inferior in various degrees.

It is unlikely that the depth of liquid in an impinger is in any way critical provided that it is sufficient to ensure that the air-jet entrains and constantly recirculates the liquid. This process washes impinged particles from the base of the flask and suspends them in the liquid. The liquid barriers presented to the escaping air by the disturbed liquid near the jet must play a part in increasing the filtration efficiency.

It is emphasized that errors due to the inefficient intake into a sampler of the larger aerosol particles with high inertia may completely swamp impingement losses. Comparative tests between different sampling systems must be carefully designed with this in mind.

Summary

The unique features of the liquid impinger as a viable aerosol sampler are described.

Sonic velocity impingement is shown to have a lethal effect on the more sensitive types of bacterial cell.

Tests on various types of impinger indicated an optimum impingement velocity of roughly 300 m.p.h. Below this figure impingement efficiency for small particles falls off, while above it lethal effects start.

Three types of impinger are shown to have high efficiency for the organisms used. Other types, including one hitherto standard, are less satisfactory.

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