Multistage Liquid Impinger

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INTRODUCTION

Instruments which collect viable airborne particles by inertial impact may be divided into two classes. Class I embraces instruments which project the viable particles straight onto the surface of a nutrient agar gel. Here they grow during incubation into one colony per viable particle, regardless of the size of the particle or the number of viable cells it contained. In class I are the slit-sampler, cascaded slit-sampler, sieve sampler, and the cascaded sieve or Andersen sampler, the features of all of which are conveniently summarized by Decker et al. (1). There is also the design of Lidwell (4). Class II samplers, with which we are concerned in this paper, project the particles into liquid, where they are broken up into their individual component cells. The liquid is serially diluted, plated out, and incubated to give a colony count and an estimation of the total viable cells in the original sample.

Typical of class II is the widely used Porton or capillary impinger (6, 14) in which a jet of air, accelerated to sonic velocity by suction in excess of 15 inches (38.1 cm) of mercury depression, impinges into liquid where small particles are collected with high efficiency. The sonic velocity jet serves also to limit the flow to a constant value.

The impinger is simple, cheap, convenient, easily sterilized, and, by the addition of a pre-impinger (5, 7), becomes an approximate simulant of the upper and lower parts of the respiratory system. Class II devices have the following features not possessed by those of class I. (i) They can cope with any high concentration of aerosol by virtue of the serial dilution process, whereas class I devices are easily overloaded. (ii) They permit counts of several different organisms from
the same sample, by means of differential counts or selective media. (iii) They are very convenient when nondecaying tracers are added as one component in an artificial aerosol. The tracer, such as a spore or radioactive element, allows the rate of decay of another component to be estimated, such as that of an organism under radiation stress. This estimation is independent of changes in the aerosol concentration. (iv) Virus aerosols can be estimated. (v) When the cascade system is used to give particle size discrimination and the aerosol of interest is highly skewed in size distribution, with many small particles and very few large ones, class I devices are subject to an error which in class II is negligible. This error arises from the shape of the cut-off curves for each stage of the system (see Fig. 3). The lower tail of the curve tends to flatten off along the abscissa, so that a few of the small particles will be caught on the large particle stage(s). In class I devices, these few particles will be recorded as large weighty particles which are in fact fictitious, but in class II their effect will be negligible in the final count of total cells per stage. Therefore, when the large particle count is of interest, it should be treated with reserve in class I devices, when there are many small particles present.

The special features of the impinger system are sometimes offset by its limitations. It is not very good with very dilute aerosols, in which it yields but a few cells widely dispersed in a large volume (10 to 20 ml) of liquid; the liquid, which is under low pressure, evaporates rather quickly and also tends to freeze in cool dry air; the violent impingement can kill delicate cells (6, 14).

It was deemed desirable to design a sampler which minimized the limitations of the Porton impinger and could be more widely used. The outcome is the subject of this paper. The new sampler has a higher sampling rate and gives a greater concentration of cells per unit volume of collecting fluid. The rates of evaporation and freezing are low and the impingement is gentle. The particle size discrimination is in three stages and is intended to simulate some of the finer details of the respiratory tract, where similar processes of inertial collection of particles operate. Also, the new sampler is more robust and more easily portable than the Porton impinger-preimpinger combination and is not subject to loss of collecting fluid through spilling as is the preimpinger. Finally, prolonged sampling periods do not result in as high a proportion of organism death as does the Porton impinger.

**Basic Description of Design**

**Standard Model**

Drawings of the form of instrument most commonly used to date are shown in Fig. 1. A and B are sectional side elevations at right angles to each other in the directions I-I and II-II, respectively. The body is entirely of Pyrex glass, the outer parallel portions being blown from thick-walled tubing of 70-mm outer diameter. The sampler has three chambers or stages, 1, 2, and 3, in serial order vertically. The air inlet tube 4 has a smoothly curved entry to promote laminar flow, a flat ground lower end, and a bore of 15 mm. The straight tube 5, also with a smoothly curved bell-mouth, a flat ground lower end, and a bore of 10 mm, is sealed into the flat floor of stage 1. This tube provides air-flow connection with the next stage. The tube 6, again with a smooth bell-mouth and bore of 10 mm, is sealed into the floor of stage 2. At its lower end, it bends and tapers smoothly and continuously to the nozzle 7, which has an internal diameter of 3.3 mm. The nozzle is close to the bottom of the annular well 8, formed as shown, and the axis of the nozzle lies in a plane tangential to the wall of the well and makes an angle of 45° to the vertical. Two circular discs, 9 and 10, made from coarse sintered glass 3 mm thick, are held about 1 mm above the flat floor of their respective chambers by the pairs of curved glass rods, 11 and 12, fused to the external walls of tubes 4 and 5, respectively. The discs 9 and 10 are twice the diameter of the bores of their respective tubes, 4 and 5, and are separated from the flat ends of these tubes by a distance equal to three-eighths of the bore. In use, the discs are constantly wetted by sampling fluid in each chamber. Access holes to each chamber are sealed by the rubber bungs, 13, 14, and 15. The lowest bung, 15, is fitted with a tube, 16, for connection to a suitable pump. This tube 16, which may be of any suitable material, projects into the center of the lower chamber and, as drawn in Fig. 1, may embody a flow-controlling critical orifice to give a constant throughput of 55 liters per min.

When operating the sampler in a cross-draft or wind, a hemicylindrical metal shield, 17, is clipped over the top so that the concave side faces upwind.

**Principles and Methods of Operation**

**First Stage**

When air is drawn through the instrument, air enters the intake tube, 4 (Fig. 1), and flows over the disc, 9, where some of the larger aerosol particles impact on the wet surface. To minimize
particle loss inside the intake tube, it should always be kept clean and dry. The ratio of 3:8 for (distance from tube to disc)/(tube bore) is that which is considered by Mitchell and Pilcher (11), and also by Mercer (9, 10), to give the steepest cut-off curve (Fig. 3), i.e., the sharpest particle-size cut-off. When the flow rate is 55 liters per min, 50% of bacterial particles 6 μ in diameter (d₀) with a specific gravity of about 1.5 will be trapped on the first sintered disc, and the proportion of particles of other sizes caught may be judged from Fig. 3. The rather high specific gravity is that measured for the dry bacterial clusters used in the calibration.

The air then flows outwards over the disc and liquid surface. It is particularly important that the sampler should be vertical and that the liquid should be nowhere higher than the upper surface of the sinters; otherwise, the outflowing film of air will encounter a standing liquid wave which will trap many particles intended for the next stage.

Second Stage

The same process of impaction and particle selection takes place at the second stage. With the smaller bore and higher velocity, the d₀ is 3.3 μ, as shown in Fig. 3.

Third Stage

The remaining particles now pass down the third tube, 6, and through the jet 7 (Fig. 1). It is important that tube 6 be smoothly tapered to the jet without any sharp bends; otherwise, particle loss on the inner walls will be excessive. For the same reason, it is important that tubes 4 and 5 should be quite smooth internally.

The tangential component of the jet direction

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**Fig. 1. Sectional elevations of standard design.**
imparts a vigorous swirl to the liquid, which ensures that impingement is always on a wetted surface, provided that the liquid volume is not less than 5 ml. The diameter of the jet was determined on the basis of the performance of the "sub-critical" impinger described by May and Harper (6), and the idea of liquid swirl came from the Shipe sampler (14).

The jet gives no more than just sufficient velocity to ensure the capture of most single bacterial cells. Tests by G. J. Harper (personal communication) established that 80 to 90% of single cells of Bacillus subtilis and Escherichia coli were retained. The design has three advantages over the standard critical orifice impinger. First, the minimal violence of the impingement minimizes or perhaps eliminates kill of delicate cells. Second, splashing and frothing are minimized, so that a high air flow can be maintained through the compact third stage chamber without liquid loss by entrainment (note that the extract tube, Fig. 1 and 2, extracts air from the center of the chamber). Third, the critical orifice or other flow control system is downstream of the extract point, so that the pressure drop on the liquid surface is only about 2 inches (5 cm) of mercury below ambient. In this way, both the rate of liquid evaporation and the possibility of freezing are very much reduced.

**Liquid Loss by Splashing**

Sometimes splashed liquid may collect on the roof of the third chamber and drip down on tube 16, whence it is entrained to waste through the tube. Such loss may be avoided by fitting the rubber drip ring seen in Fig. 2A.

**Liquid Loss by Evaporation**

If a long-period sample is taken, evaporation of the sampling fluid will occur. Evaporation is permissible to about 5 ml per stage, and the gap under the sintered discs will ensure circulation of liquid so that the disc remains moist. If evaporation threatens beyond this level, the lost liquid may be made up. With air at normal living conditions, topping-up may be necessary every 0.75 hr or so. About the same quantity will be lost from each of the three stages.
Sampling in Still Air

When the air to be sampled is calm or nearly so, as in a room, the sampler is used as it stands (Fig. 2). The smooth entrance, large diameter, shortness and smooth straight bore of the entry tube, 4, will ensure a high efficiency of collection of airborne particles.

Sampling in a Crosswind

In this case, the shield, 17, is fitted to stop cross-flow above the entry tube, thus approaching the still-air conditions. The system, which might be termed “stagnation-point sampling,” is an entirely different concept from “isokinetic sampling” where air enters a knife-edged sampling tube with no change of speed and direction from that of the wind. Obviously, isokinetic sampling cannot be achieved from a horizontally moving air stream with impaction onto a horizontal liquid surface, as a 90° turn must intervene. In fact, true isokinetic conditions cannot be achieved outside a laminar-flow wind tunnel because of air turbulence. In stagnation-point sampling, the requirement for high intake efficiency would seem to be that the intake zone should be large compared with the particle’s “stopping distance.” The stopping distance is the distance of projection of the particle into still air from a given initial velocity, and varies as the square of the particle diameter and the velocity of projection. The validity of this concept is discussed below in the Calibration and Testing sections.

Sampling from a Tube

It may be required to withdraw aerosol samples from a chamber via a tube. In such a case, the connecting tube must never be inserted into the intake tube of the sampler by means of a bung, etc., as the jet effect from the narrow tube would completely alter the collection characteristics of the first stage. Ideally a 2.75-inch (7-cm) bore rubber hose would be pressed over the whole of the top of the sampler, maintaining the same wide bore to its source. If this is difficult, a tube at least as wide as the intake tube over the whole of its length should be used for the connection, with a wide metal flanged piece at its end. A sponge-rubber ring should be fitted under the flange, and the whole should be pressed firmly on top of the sampler.

Filling with Sampling Fluid

Fluid suitable for the organisms of interest is pipetted into each stage through the bung holes. The two upper stages are filled until the liquid surface is just below the upper surface of the sintered disc when the sampler is standing vertically. The upper surface of the disc will always be wet by capillarity, provided that the sinter is maintained in a grease-free condition by appropriate cleaning procedures. The standard model requires between 7 and 10 ml, varying from sampler to sampler, because of the vagaries of glass-blowing. It is convenient to mark indelibly by the side of each bung hole the volume required, as shown in Fig. 2A. Into the lower stage, 10 ml is pipetted.

Emptying

After use, the sampler may be shaken gently to mix the liquid, which is then withdrawn by a 10-ml calibrated pipette, to measure the volume. The liquid should be squirted a few times over the sintered disc to ensure removal of all organisms. This, and subsequent pipette mixing, must be done vigorously to ensure breakdown of clumps into single cells.

Sterilization and Cleaning

Before autoclaving or heat-sterilizing the sampler, the rubber bungs must be removed. When collecting fluid containing dissolved solids has been used, the sintered discs should be washed thoroughly with distilled water before heating, so that solids do not get baked into the pores. The sintered glass must always be kept chemically clean.

Flow-rate Control

Any system of flow control may be used, but the Hartshorn or venturi-shaped critical orifice (16 in Fig. 1) is perhaps the most convenient. These orifices constrict in a smooth curve to the throat, and then expand at an included angle of 4° over a length of about 1 inch (2.5 cm). As pointed out by Druett (2), this orifice geometry allows critical flow to commence at a very small depression, 4 to 5 inches (10.2 to 12.7 cm) of mercury compared with 15 inches (38.1 cm) for a conventional parallel-sided orifice. A substantial economy of pumping power is thus afforded. The addition of the 2-inch pressure drop across the sampler gives a safe minimum of only 7 inches of mercury depression required from the pump at the full flow rate of the sampler. Critical orifices are made slightly undersize and thenreamed out until, in situ in the sampler, the desired throughput is achieved. In the present case, the initial bore is 2.5 mm for final adjustment to 55 liters per min. Systems of control not embodying a critical orifice are (i) a flowmeter with valve, downstream of the sampler; (ii) a compressed air or steam ejector operating at a constant pressure.
(although these can only give a small pressure drop on their suction side, it is adequate for the 2 inches of Hg that this sampler requires); (iii) a sensitive pressure gauge just downstream of the sampler followed by a valved T-piece in the pump suction line. The valve is adjusted to bleed ambient air into the line until the depression across the sampler is that which in prior calibration gave the correct flow. The latter system makes the least demands of all on the pump but must be watched to maintain steady flow unless a sensitive pressure-regulated bleed valve is built into the T-piece.

**Other Features of the Design**

**Wet-disc Collection Surfaces**

The disc surfaces are moist, as are particle deposition areas in the respiratory system. This similarity is important in that the retention capability of a surface undoubtedly depends on its physical state as well as on the physical state of the surface of the particle.

Another valuable feature of the sintered discs is that their geometry, hence, impaction efficiency, is unaffected by the gradual evaporation of the liquid surface. Cells remain viable on the moist surface when the optimal collecting fluid is used, permitting prolonged sampling.

**Portability**

The sampler has been designed so that it has a smooth external surface with no projecting glassware which might be damaged by chance knocks. The rubber bungs and rubber-mounted suction tube act also as fenders, and more such protection could be added if desired. The thick-walled glass tubing has considerable mechanical strength, provided that the whole has been correctly annealed. The sampler will stand up to prolonged usage and repeated sterilization when reasonable care is employed and use is made of properly fitted storage boxes for transportation. It will stand firmly on a bench of its own accord but will not resist being dropped on a hard surface.

**Nonspill Property**

When the sampler is charged with sampling fluid and has the bungs in place, liquid cannot be spilled or transferred from one stage to another, however much the sampler is turned or inverted. The principle is similar to that of the unspillable ink-well.

**Variations of the Basic Design**

The basic design may easily be varied. In addition to the standard 55 liter per min model (A in Fig. 2), two other models have been constructed (B and C in Fig. 2).

The 20 liter per min model (B) has stage characteristics somewhat different from A. Its first stage was designed to collect "fall-out" particles and has a 50% cut-off at 10 μ; the second stage has a similar cut off to the standard "pre-impinger" (50% at 4 μ), as described by May and Druett (5), so that its collection resembles upper respiratory retention, whereas the third stage resembles lower respiratory retention. The first-stage tube bore is 15 mm, and the jet impulse is so slight that no sintered disc is needed as the liquid surface remains undisturbed by the flow; nor is any filling hole required in the top stage as filling and emptying can be performed through the entry tube. A small dent in the glass floor of the top stage immediately under the entry tube acts as a sump. The clearance between the liquid surface and the bottom of the entry tube is 4 mm. The change in this as the liquid evaporates is unimportant. The second-stage jet diameter is 7.5 mm and the third, 2 mm. Each stage holds 4 ml of sampling fluid.

The small model (C), which is only 3.5 inches (8.9 cm) high, is operated at 10 liters per min, and was designed to have the same particle size range per stage as the large model (A). The jet bores are: first stage, 8 mm; second stage, 5 mm; third stage, 1.42 mm. The ratio of disc diameter to jet bore and the ratio of the clearance between the disc surface and the end of the tubes is as already described. This model is very much more compact than the standard Porton impinger which functions at about the same flow rate, yet it is capable of yielding more information and has the other advantages of the new design. It holds 2 ml of sampling fluid per stage.

**Calibration and Testing**

**Particle Intake Efficiency**

Wind-tunnel tests on the sampler were carried out by J. Edwards (personal communication). With the hemicylindrical baffle in place (17 in Fig. 1), the sampler was exposed to wind-borne aerosols of dyed, involatile, and uniform droplets generated by a spinning-top atomizer (8). An array of knife-edged isokinetic orifices around the sampler measured the absolute dosages. Estimations were by colorimetry.

Table 1 shows that the intake efficiency with the baffle in place is high, except for the largest particles and highest wind speed. The latter figures can be greatly improved by employing a larger baffle. For example, with no baffle, the intake efficiency was found to be only 9.6% at 15 μ and 10 mph but was raised to 99% by using...
MULTISTAGE LIQUID IMPINGER

TABLE 1. Per cent intake efficiency*  

<table>
<thead>
<tr>
<th>Drop size</th>
<th>Wind speed</th>
<th>2 mph</th>
<th>5 mph</th>
<th>10 mph</th>
<th>15 mph</th>
<th>20 mph</th>
</tr>
</thead>
<tbody>
<tr>
<td>μμ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>93 (0.3)</td>
<td>106 (0.7)</td>
<td>87 (1.5)</td>
<td>73 (2.2)</td>
<td>64 (2.9)</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>102 (0.6)</td>
<td>102 (1.6)</td>
<td>69 (3)</td>
<td>61 (4.5)</td>
<td>57 (6)</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>98 (1.1)</td>
<td>89 (2.8)</td>
<td>56 (5.6)</td>
<td>39 (8.3)</td>
<td>27 (11)</td>
</tr>
</tbody>
</table>

* Particle stopping distance (in millimeters) is given in parentheses.

Fig. 3. Individual stage cut-off curves (solid lines) and relative distribution of particles among all stages (dotted lines).

a 6-inch square concave baffle, compared with the efficiency of 69% in Table 1, obtained with the Fig. 1 baffle. It is clear that a baffle is an essential addition to the sampler when there is a wind or cross draft. Note also that in Table 1 there is a good inverse correlation between the stopping distance (given in parentheses) and the intake efficiency, as predicted. With the particular conditions of the Table 1 tests, the intake efficiency is less than 50% when the stopping distance is greater than the radius of the intake tube.

Stage Cut-off Curves

It is essential to know the performance of each stage in terms of particles of each size retained; one must also know the relationship of this parameter to the dimensions of the jets and the throughput of sampled air. For this work, dry spherical airborne particles, nearly uniform in particle size, were generated from solutions of the intense blue dye Chlorazol Sky Blue (Imperial Chemical Industries Ltd.) sprayed from a spinning-top atomizer (8). The atomizer was mounted in a vertical wind tunnel similar to that described by Druett and May (3). In this, the up-flow of air permits the rather large droplets generated to dry down to their final size without serious wall loss. The size of the dry particles is determined by the initial wet droplet size and the concentration of the dye solution. In this way, the range 2.5 to 10 μ was studied in small intervals, the relative proportion of particles caught in each stage being obtained by colorimetric estimation of the dye collected in the water with which the stage was filled. The spherical dye particles had about the same density as the particles obtained by spraying suspensions of bacterial cells (ca. 1.5 g/cc). The results for the standard 55 liters per min model are shown in Fig. 3, where the continuous lines are the cut-off curves for each stage considered individually, and the dotted lines (which in parts coincide with the continuous lines) show the percentage of all particles entering the sampler collected by each stage.

The cut-off curves of Fig. 3 are not as steep as those presented for the same optimal geometry by Mitchell and Pilcher (11) and by Mercer (9). There are two possible reasons for this. First, their methods were different, giving aerosols...
which may have been more truly homogeneous in particle size than those used in the present work, where a spread in particle size tends to flatten the cut-off curves and reduces the precision. Second, the flow in the two upper chambers is rather complex. The air, after flowing out over the sintered discs, passes over a rather extensive liquid surface, and then turns upwards and inwards to enter the next jet tube. This causes turbulence and vortices which could throw out particles, though wall losses have not actually been noted other than to a limited extent in the tubes. Although the cut-off curves are less steep than hoped for, they are in fact as sharp as those of the preimpinger (5, 7) and the cascade impactor, and certainly not less sharp than the parts of the respiratory system that the sampler could simulate.

**Effect of Varying Jet Sizes and Flow Rate**

Workers wishing to adapt performance to their own requirements, with jet sizes or flow rates different from those described here, may use Fig. 4 as a guide. The chart derives from the expression for the dimensionless impaction parameters \( P = k \rho V d^2/\eta l \), where \( k \) is a constant which in practice varies from system to system, \( \rho \) the particle density, \( V \) the jet velocity, \( d \) the particle diameter, \( \eta \) the gas viscosity, and \( l \) the jet diameter (or characteristic length). For a given efficiency of impaction of particles, \( P \) is constant, and in the present case we are concerned with its value at \( d_{50} \), the 50% cut-off diameter. For operation in air at normal temperature and pressure, we can also take \( \rho \) and \( \eta \) as constants so that, as in Fig. 4, we can relate \( d_{50}, l, \) and \( V \) (transformed into the more convenient flow rate parameter). The foundation of Fig. 4 was the set of six values for \( d_{50} \) \( (v/l)^{1/2} \) obtained from the calibration, as described in the preceding section, of the top two stages of the three models shown in Fig. 2. The values were 113, 108, 105, 122, 118, and \( 122 \times 10^{-4} \) calculated in units of centimeters and seconds.

For any given value of \( d_{50} \), the shape of the cut-off curve will be geometrically similar to those of Fig. 3.

**Testing with Bacterial Aerosols**

To study the performance of the sampler under rigorous conditions, a procedure identical to that previously described (6, 7) was adopted. In the relatively warm and dry conditions of summer daylight, a mixed suspension in known ratio of vegetative organisms (E. coli), which lose viability rapidly under such conditions, particularly in full sunlight, and of nondecaying spores (B. subtilis var. niger) was sprayed by a pneumatic hand spray and sampled at sufficient distance downwind for appreciable cell death to have taken place. By comparing the ratio of the two components recovered from the several stages of the sampler with their ratio before spraying, the percentage of E. coli cells remaining viable could be determined.

In the sampling array, two pairs of samplers were employed, each shielded from direct light. Each pair consisted of a three-stage glass sampler and a “tilting” preimpinger plus impinger unit (7). The latter unit was known to have a high efficiency of intake of aerosol particles and was used as a standard reference sampler for comparison with the three-stage sampler. The two pairs were within a few yards of each other, close enough to ensure that they were exposed to comparable, but not necessarily the same, dosages.
<table>
<thead>
<tr>
<th>Test no.</th>
<th>Length of time airborne</th>
<th>&quot;Tilting&quot; sampler's total dosage</th>
<th>Three-stage sampler</th>
<th>Percentage of E. coli remaining viable</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(Escherichia coli)</td>
<td>(Tracer spores)</td>
<td>[Dosage of E. coli cells]</td>
</tr>
<tr>
<td>1</td>
<td>60 sec</td>
<td>22</td>
<td>76</td>
<td>14.3</td>
</tr>
<tr>
<td>2</td>
<td>70 sec</td>
<td>374</td>
<td>1,700</td>
<td>199</td>
</tr>
<tr>
<td>3</td>
<td>65 sec</td>
<td>398</td>
<td>1,745</td>
<td>199</td>
</tr>
<tr>
<td>4</td>
<td>55 sec</td>
<td>259</td>
<td>1,559</td>
<td>199</td>
</tr>
<tr>
<td>5</td>
<td>3 min</td>
<td>754</td>
<td>4,070</td>
<td>199</td>
</tr>
<tr>
<td>6</td>
<td>50 sec</td>
<td>350</td>
<td>3,360</td>
<td>199</td>
</tr>
<tr>
<td>7</td>
<td>4.5 min</td>
<td>2,550</td>
<td>8,940</td>
<td>199</td>
</tr>
<tr>
<td>8</td>
<td>60 sec</td>
<td>1,589</td>
<td>6,520</td>
<td>199</td>
</tr>
<tr>
<td>9</td>
<td>3.5 min</td>
<td>202</td>
<td>1,192</td>
<td>199</td>
</tr>
</tbody>
</table>

- "Dosage" in units of cells. Minutes per liter is the total number of cells in the sample divided by the sampling rate.
- The three-stage sampler recorded in the second line of each test was allowed to run for 30 min after receiving its sample. The other three samplers in each test ran for only about 5 min.
- The recorded tracer spore dosages have been adjusted to be equivalent to a starting ratio of unity for concentration of viable E. coli/tracer in the sprayed suspension.
- Column 13 is the percentage of column 5/column 9, and so on.
- Pump failed.
TABLE 3. Atmospheric conditions during field tests of Table 2

<table>
<thead>
<tr>
<th>Test no.</th>
<th>Temp</th>
<th>Humidity (%)</th>
<th>Illumination perpendicular to sun (ft·c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17.8</td>
<td>56</td>
<td>8,000 (clear sky)</td>
</tr>
<tr>
<td>2</td>
<td>18.9</td>
<td>48</td>
<td>2,000–8,000 (5/10 cloud)</td>
</tr>
<tr>
<td>3</td>
<td>19.4</td>
<td>60–65</td>
<td>800–2,000 (overcast)</td>
</tr>
<tr>
<td>4</td>
<td>16.1</td>
<td>68</td>
<td>1,500 (overcast)</td>
</tr>
<tr>
<td>5</td>
<td>18.9</td>
<td>57–61</td>
<td>2,000–8,000 (5/10 cloud)</td>
</tr>
<tr>
<td>6</td>
<td>17.2</td>
<td>53</td>
<td>2,000–7,500 (5/10 cloud)</td>
</tr>
<tr>
<td>7</td>
<td>15.6</td>
<td>58</td>
<td>2,000 (overcast)</td>
</tr>
<tr>
<td>8</td>
<td>18.3</td>
<td>54</td>
<td>8,000 (clear sky)</td>
</tr>
<tr>
<td>9</td>
<td>19.4</td>
<td>54</td>
<td>8,000 (clear sky)</td>
</tr>
</tbody>
</table>

The first pair of samplers was switched off after sampling the aerosol for a few minutes, and in the second pair the three-stage sampler was left running for 0.5 hr to test whether the continuous aspiration of the dry air over the collected particles affected their viability. During this time, about 40% of the sampling fluid (phosphate buffer plus 1 M sucrose plus antifoam) evaporated.

Results

The results from nine tests are summarized in Table 2. Each test has two lines of dosage figures, one line for each pair of samplers. Table 3 gives the atmospheric conditions of the tests.

Effect of running for 0.5 hr. This may be judged by comparing the ratios of total dosages measured by each type of sampler in the first pair (top line of each test) with corresponding ratios in the second pair (bottom line) after extended operation of the three-stage sampler. If the extended operation caused a loss of viability, the ratio in the second pair would be less, on the average, than in the first. These ratios have been extracted, and are presented in Table 4 for the E. coli and in Table 5 for the spore tracer. Although in both tables the means from the second pair are slightly lower numerically, statistically they do not differ significantly from each other in either pair. Neither organism, therefore, shows appreciable loss after 30 min of aspiration in the conditions of these tests.

Overall recovery of viable cells. The intake and yield of viable cells in the three-stage sampler as compared with the tiling unit may also be judged from Tables 4 and 5. Means C and D in Table 5 are not significantly different from unity for the very robust spore tracer. We may conclude from this that both types of sampler in the pairs have the same intake efficiency and subsequently yield the same dosage of viable cells. In Table 4, the means A and B are significantly greater than unity, indicating that the three-stage sampler yields more viable cells of E. coli. This is due to the absence in the three-stage sampler of the violent sonic velocity impingement in the Porton impinger, which is known to cause death of sensitive cells (6, 14). The tests do not tell us anything about the absolute recovery of E. coli, as one cannot assert from the figures presented that cells are not being killed in both samplers. However, in recent work by K. P. Norris (personal communication), the three-stage sampler in some winter conditions of less light, higher humidity, and long aspiration times has yielded viability figures for E. coli in the region of 100% on all stages. This indicates that there was no killing effect in the sampler. It is reasonable to conclude from accumulated experience with various other types of sampler, as well as with the new sampler, that neither the systems of impaction nor the washing-off process from the sintered discs results in appreciable loss of viable cells. Nevertheless, workers proposing to use the sampler with very delicate organisms would be well advised to carry out viability tests under their experimental conditions.

Effect of particle size on viability. As one of the main functions of this sampler is to yield estimates of organism viability within the various particle size classes, it is of value to examine the information in columns 13, 14, and 15 of Table 2, relative to this point. The periods during which the cells were airborne in the daylight was either close to 1 min or 3 to 4.5 min (column 2). The percentage of E. coli cells remaining viable after these periods has been averaged in Table 6. After 1 min, the small particles contained only half the proportion of viable cells that the large ones did. In the longer period, the small particles have continued to die off more rapidly, to about one-fifth the viability, on the average, of the large particle figure. In the full sunlight of the final test recorded in Table 2, nearly all the E. coli cells in small particles succumbed in 3.5 min.

Discussion and Conclusions

Morrow (12) has reviewed the recent position on the relationship of particle size to respiratory deposition. He stresses the importance of selective sampling, which is usually designed to accept the small, lung-attacking particles and to reject the harmless large ones. For infective aerosols, selective sampling seems even more important because of two factors. First, it has been shown in the laboratory that with many infective diseases the smallest particles are the most dangerous, but it is also considered that in those infections which start in the upper respiratory tract,
TABLE 4. Ratios of total Escherichia coli dosage in three-stage sampler to total in tilting sampler

<table>
<thead>
<tr>
<th>Pair</th>
<th>Test no.</th>
<th>Geometric meansa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>0.89</td>
<td>1.64</td>
</tr>
<tr>
<td>2</td>
<td>1.48</td>
<td>1.04</td>
</tr>
</tbody>
</table>

* Means A and B do not differ significantly from each other, but each is significantly greater than unity.

TABLE 5. Ratios of total tracer spore dosage in three-stage sampler to total in tilting sampler

<table>
<thead>
<tr>
<th>Pair</th>
<th>Test no.</th>
<th>Geometric meansa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>1.17</td>
<td>1.22</td>
</tr>
<tr>
<td>2</td>
<td>0.86</td>
<td>1.01</td>
</tr>
</tbody>
</table>

* Means D and D are not significantly different from unity.

or in open wounds, etc., large particles are the most dangerous. Second, as Table 6 indicates, maintenance of viability in the airborne state may be markedly dependent upon the size of the airborne particle.

We therefore need to know the airborne concentration and state of viability of cells in various particle size ranges. It is not necessary that these ranges be narrow, sharply defined, and many in number, because physiological response to particle size effects cannot have sharp boundaries. For example, all sizes of particles can probably infect a wound, though large ones are much more likely to fall out than small ones; particles of say 6 to 7μ are found in the alveoli, but those of 1 to 3μ can penetrate in proportionately much greater numbers; persons differ in structure and breathing patterns, and flow rates vary widely. A selective sampler can, therefore, do no better than give a performance resembling a human average which can be generally accepted. The present model attempts this in its selection of three ranges: "large" particles, i.e., those greater than 6μ in diameter (ρ = 1.5) which are normally retained in the upper respiratory tract; an intermediate range of 3- to 6-μ particles which are likely to lodge in the bronchi or bronchioles; and the fine range, 3μ to single cells, which penetrate to the alveoli. Probably many bacterial particles are hygroscopic and will have time to increase in size in the depths of the lung. Lung retention of 1-μ viable particles should therefore be much higher than it is for dusts, many of which are expired at this size, so that the retention of 80 to 90% of single cells (E. coli) given in Fig. 3 seems reasonable. Single virus particles, ca. 0.3μ and smaller, would not be retained in the sampler, whereas in the lung a high proportion would be retained by another process, diffusion to the walls. It seems unlikely, however, that such small isolated virus units can ever be generated in quantity by natural processes in air. The writer knows of no facts to confirm or refute this important point, which may be extremely difficult to resolve.

It is not claimed that the size ranges of the new sampler are necessarily the best. Further knowledge may require them to be altered in cut-off or in number, or both, and this can readily be done by use of the data in Fig. 4. The standard design might be modified by adding a top stage with similar characteristics to the top stage of model B, Fig. 2. This could distinguish large wound-infecting particles from respirable ones. In other work, a simple two-stage "upper" and "lower" respiratory tract simulation, as in stages 2 and 3 of model B, might be quite adequate. Selective sampling is based on the mass of work done on the effects of toxic industrial dusts on the relatively few workers exposed to them, whereas much less seems to be known about the equivalent

TABLE 6. Means of results from Table 2, columns 14, 15, and 16

<table>
<thead>
<tr>
<th>Stage</th>
<th>Percentage of Escherichia coli viable at 1 min airborne</th>
<th>at 3 min plus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top</td>
<td>38</td>
<td>29</td>
</tr>
<tr>
<td>Middle</td>
<td>27</td>
<td>21</td>
</tr>
<tr>
<td>Bottom</td>
<td>19</td>
<td>6</td>
</tr>
</tbody>
</table>
properties of the causative agents of airborne infection, to which all members of society are exposed. It is hoped that the new sampler, modified if necessary and used perhaps in conjunction with those of class I, will extend the latter field of knowledge.

The usefulness of the sampler is not necessarily confined to viable aerosols. It might well be used in air pollution work, indoors and out, and anywhere where respiratory irritation is related to particle size.

Finally, it may be mentioned that the gap between class I and class II samplers may be bridged by using soluble gel collection surfaces (gelatin with glycerol), which may afterwards be washed off in warm water for dilution and plating to give a single cell count. Both the Andersen (1) and Battelle (11) impactor configurations can easily be converted to this use. Studies on these lines were carried out in the present work, and were quite promising, except that the problem of finding a sufficiently stiff yet soluble gel to resist the high velocity impingement in the final stage was not solved. Microscope studies showed that impinged cells could not be effectively washed off agar surfaces, and dry surfaces cannot be used because of death of cells by desiccation. Also, the preparation of gel surfaces requires extra work, and it was concluded that the glass-liquid model is preferable because it offers greater simplicity in use, gives a reliable performance, and is inexpensive to manufacture.

**Availability**

The sampler may be obtained from A. W. Dixon and Co., England. The design is patented (No. 1964 65) by the British Government.

**Acknowledgments**

I am greatly indebted to W. I. P. Nelson for his skillful work in making the prototype samplers, to G. J. Harper and his staff for the assay figures recorded in Table 2, to J. Edwards and his staff for providing the data for Table 1, to B. R. D. Stone for measurements, with a Millikan cell technique, of the densities of the dry particles of bacterial clusters and of the blue dye used in the tests, and to S. Peto for statistical tests.

**Literature Cited**