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The control by ventilation of airborne bacterial transfer between hospital patients, and its assessment by means of a particle tracer

I. An airborne-particle tracer for cross-infection studies

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SUMMARY

A simple and convenient particle tracer for studies of the effectiveness of isolation units and other places in limiting the airborne transfer of bacteria is described. Particles of potassium iodide 7–8 μm. diameter are generated by spraying from solution and collected on membrane filters. The particles can be identified by development with 0·1 % acid palladium chloride solution, when dark brown spots approximately 100 μm. in diameter are produced.

INTRODUCTION

Although the relative importance of the airborne and contact routes for the transfer of infection is still uncertain, isolation units are constructed with elaborate and expensive ventilation systems designed to eliminate the transfer of airborne micro-organisms between patients. It is, moreover, common experience that many such installations do not function in accordance with their design specification.

It is possible (Lidwell, 1972), by making some simplifying assumptions, to estimate the capability for air isolation of a particular design. In order to check the actual performance of a unit under working conditions some form of airborne tracer must be used. Identifiable strains of micro-organisms carried by the patients may be used for this purpose (e.g. Williams & Harding, 1969), but although such a method is closer to the real issue than is the use of gas or inert particulate tracers it has several limitations. The dispersing sources are not under control, and the relatively small numbers of identifiable airborne particles dispersed means that the method is rather insensitive and that observation must be carried out over a prolonged period.

Nitrous oxide has been used as a gaseous tracer (e.g. Lidwell & Williams, 1960; Baird, 1969), but the instruments available for measuring it are of limited sensitivity, and determination to an accuracy of 10 % requires concentrations of the order of $1/10^4$ in air. Any higher sensitivity would often lead to difficulties of interpretation in a hospital because of the widespread medical use of this gas. Much greater sensitivities can be obtained by the use of ion-capture detectors to estimate low concentrations of some halocarbons and similar compounds (N. Foord

& O. M. Lidwell, to be published). Estimations to within 10% can be obtained at concentrations as low as $1/10^9$ with some compounds, and the use of a column for chromatographic separation also allows more than one tracer gas to be estimated at one time, thus allowing simultaneous study of transfer from several source positions.

Airborne micro-organisms are however necessarily particulate and the particles responsible for transferring bacteria through the air are generally large enough to have a settling velocity in air (Noble, Lidwell & Kingston, 1963) of the order of 0·3 m./min. This may lead to substantial quantitative and perhaps qualitative differences in the pattern of dispersion of these particulates compared with the dispersion of a gaseous tracer. In addition, if there is any recirculation in the system, gases will not be retained in the filters, and no estimate of particulate dispersion can then be made by gas tracer studies. A satisfactory particle tracer would therefore be a useful tool for assessing the performance of an isolation system.

Fluorescent particles have been used as particulate tracers and an elegant method has been devised by Goldberg (1968) for distinguishing the tracer particles from other kinds of airborne particles that may produce spurious signals in the detecting equipment. However, although this system is very sensitive and has the advantage of giving essentially instantaneous readings, the equipment is elaborate and does not readily lend itself to simultaneous estimates of the concentration of particles at many sites at one time. Bacterial tracers have been widely used in some situations (e.g. Lidwell & Towers, 1969), but there are objections to the widespread dissemination of any micro-organism, no matter how apparently innocuous, in a working hospital.

The ideal particulate tracer would be imperceptible, innocuous, easy to produce and disperse in a range of particle sizes, and such that individual particles are easily recognized and counted after collection.

DEVELOPMENT OF A METHOD

Particles of a controlled size are perhaps most easily produced in the numbers required by spraying a solution or dispersion of the selected substance in a volatile solvent. Simple air-driven sprays, or humidifying devices in which the liquid is thrown from a revolving disk against a set of stationary teeth, produce droplets with a wide size range and the particulate cloud is therefore inhomogeneous. The sizes of the particles carrying airborne micro-organisms also vary over a wide range but the degree of inhomogeneity is not necessarily the same as that of the sprayed droplets and variations in size make the detection and accurate counting of the particles more difficult. Spinning disk sprays, driven either by air or electrically and capable of producing a homogeneous cloud of droplets down to diameters of 40 μm. or less, are commercially available.

Our first experiments were carried out by spraying aqueous solutions of sodium chloride. When the resulting particles were collected by impingement or settling on Petri dishes filled with 0·5% agar (Oxoid Ionagar) containing silver nitrate the

Table 1. *Spot sizes obtained by collecting sodium chloride particles on* 0·5 % *ion agar containing silver nitrate, spot diameters in micrometres*

Conc. NaCl sprayed (%)	Particle diameter (μm.)	Conc. $AgNO_3$ (%)				
		1	0·5	0·1	0·01	0·001
8·3	10·3	—	—	135	—	—
2·5	7·6	46	58	98	180*	250†
0·3	3·7	—	—	44	—	—

* These spots were rather faint.

† These spots were very faint. Spots could not be detected if the silver nitrate concentration was below 0·001 %.

resulting approximately hemispherically shaped deposits of silver chloride could be converted by exposure to light, preferably long-wave ultra-violet, or by treatment with very dilute photographic developer, into well-defined nearly black disks. Table 1 shows the diameter of the resulting spots for different concentrations of silver nitrate and three sizes of sodium chloride particles. Although samples of air collected outside the laboratory contained fewer than one sodium chloride particle apparently 5 μm. or larger, per 50 l. the numbers found inside the building were 20–30 times greater, so that sodium chloride is not a suitable tracer for use within occupied buildings, nor can silver nitrate agar be used for the detection of other halides. Particles of water-soluble iodides can however be detected by other methods, in particular the reaction with palladous chloride, without interference from chloride.

Potassium iodide as a tracer particle

Potassium iodide particles, produced by spraying a solution of the salt, are collected by drawing the air to be sampled through a membrane filter. The particles are made visible by treating the membrane with a solution of palladous chloride. If prepared under suitable conditions the membranes when dried are stable and can be stored for long periods. The best spots were obtained by laying the membranes carefully onto the surface of the palladous chloride solution. The membrane then wets through rapidly. The spots of dark brown insoluble palladous iodide form almost instantaneously and the membranes can be lifted off and placed on absorbent paper to dry. A rinse in water before drying improves contrast by removing the excess palladous chloride and a large proportion of any dust particles which have been collected. The diffusion conditions on the surface of a membrane filter are not so well defined as on an agar plate and the resulting spot size is variable and not so simply related to the size of the particle and the concentration of the reagent as it is with the sodium chloride particles described in Table 1. Table 2 shows the average spot sizes obtained with potassium iodide particles of 7 μm. diameter, prepared by spraying a 5·6 % aqueous solution of the salt from a 25 mm. diameter spinning disk rotating at approximately 1000 rev./sec. As the size of the potassium iodide particle was reduced the diameter of the developed spot became only slightly smaller but its colour became fainter and its edges less distinct and spots arising from particles smaller than 3·5 μm. were very difficult to identify.

Table 2. *Spot sizes obtained by collecting potassium iodide particles (7 μm. diameter) on a membrane filter* and developing with palladous chloride solution, spot diameters in micrometres*

Conc. $PdCl_2$ (%)			
1	0·5	0·2	0·1
60	70	90	95

* Millipore SSWP 02500.

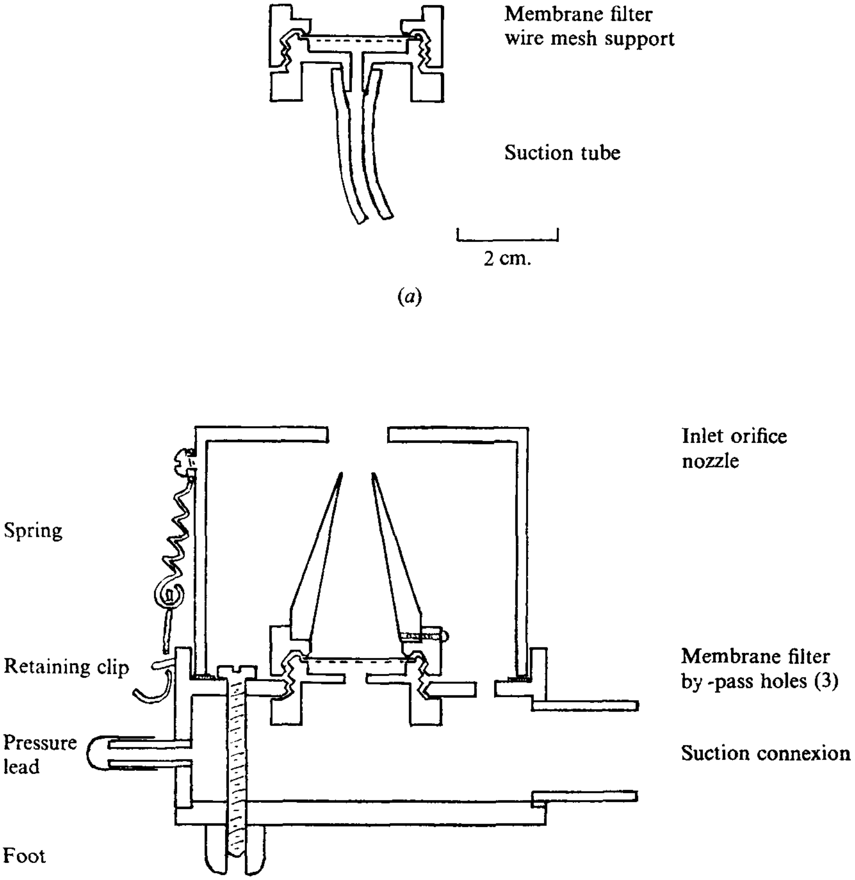
The grade of membrane filter used had little or no effect on the spot size but the spots were better defined the smoother the surface of the membrane.

The palladous chloride must be dissolved in an acid solution – N/10 hydrochloric acid is convenient – and should be filtered if cloudy. It keeps indefinitely, although after much use it may be necessary to filter it. The best concentration to use depends on the particle size of the potassium iodide. Denser colour but smaller spots are obtained with the stronger solution, so that smaller particles are visible after development. We have been interested in particles of 7–8 μm. diameter, with a settling velocity in air of approximately 0·3 m./min., corresponding to the median diameter commonly observed for airborne bacteria-carrying particles in the hospital situation. For particles of this size a 0·1 % solution gives good spots nearly 100 μm. in diameter, which can be easily seen and counted with a low-power (× 10) binocular plate microscope. At this power the field of view can be more than 20 mm. diameter, which is enough to cover the whole of the collecting surface of a 25 mm. membrane filter. Plates 1 (*a*) and 1 (*b*) show the spots as seen under a counting grid. Those in Plate 1 (*a*) are derived from particles of uniform size, approximately 8 μm. in diameter, obtained by spraying a 3·5 % solution of potassium iodide in 80 % ethyl alcohol from a spinning disk similar to that referred to above. Alcoholic solutions are usually preferable since it is then easier to ensure uniform wetting of the disk. The spots are relatively uniform in size and easy to identify. Plate 1 (*b*) shows spots derived from the particles produced by an air-driven baffled spray with approximately the same median diameter. The wide range of sizes leads to great variability in the visibility of the spots and it is difficult to establish a cut-off point for counting.

Collection of particles

The simplest method is to use a small plastic membrane filter holder, e.g. the Gelman easy pressure filter holder with the cone on the inlet side machined away (Fig. 1 (*a*)). With simple filter holders of this kind the rate of sampling is limited by the resistance of the filter. This varies with pore size and with different makes and batches of membranes. At 20 cm. water pressure the flow through a 25 mm. diameter filter in its mount varies from not much more than 1 l./min. for a pore size of 0·8 μm. to nearly 4 l./min. at a pore size of 5 μm. We have generally used a pore of size 3 μm. (Millipore Filter Corporation, Bedford, Mass., U.S.A., ref. SSWP 02500) which gives a flow rate around 3 l./min. at the above suction pressure.

Increased sampling rates can be obtained by the use of a centripetal sampler of the kind described by Hounam & Sherwood (1965). A single-stage version only is



(*a*)

(*b*)

Fig. 1. (*a*) Section of plastic filter holder (Gelman Instrument Co., Ann Arbor, Michigan, U.S.A.) adapted for air sampling. (*b*) Centripetal sampler: orifice diameter, 1·25 cm. (½ in.); nozzle diameter, 0·62 cm. (¼ in.); orifice nozzle spacing, 0·62 cm. (¼ in.); outer cone angle, 20° + 20°; by-pass holes (3), 0·41 mm. (0·16 in.) diameter. Cone and cap of brass, body of aluminium alloy, plastic filter holder the same as (*a*).

required and a convenient form of the device is shown in Fig. 1(*b*). With the dimensions given, a sampling rate of 100 l./min. is reached with a suction pressure of 20 cm. of water but only 3 l./min. passes through the filter. Inertia, however, carries a high proportion of particles above a certain size in the sampled air into the axial flow from which they are collected by the filter. Langmead & O'Connor (1969) have given a theoretical analysis of this type of sampling system. For the geometry illustrated in Fig. 1(*b*) near 100% collection would be achieved for particles of potassium iodide (density 3·1) above 5·5 μm. in diameter at a sampling rate of 100 l./min., and 50% collection of particles 3·7 μm. in diameter. For particles of 7 μm. in diameter 50% collection efficiency is reached at a flow of 28 l./min. and near 100% at flows above 63 l./min. Experimental observations largely confirmed these calculations. Efficiencies of 80–100% were obtained with particles approximately 7 μm. in diameter at flows of 60 l./min. or more. Below this flow rate the

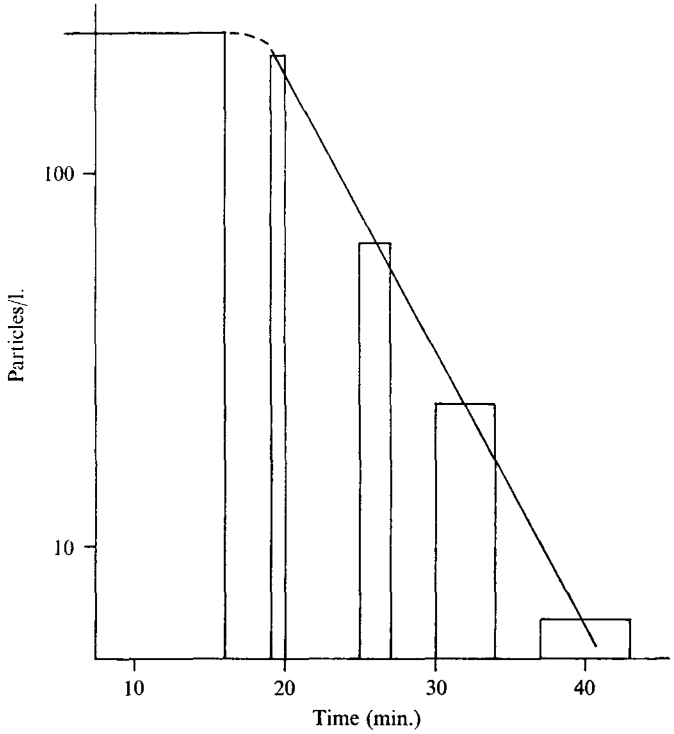


Fig. 2. Die-away rate of 7–8 μm. potassium iodide particles in patient room. The height of each column indicates the average concentration of particles during the sampling period. Spraying was discontinued at the end of the 16th minute.

efficiency appeared to fall off rather more rapidly than calculated to around 20 % at a flow rate of 30 l./min. Failure to collect very small particles is advantageous in practice. Potassium iodide particles below 3·5 μm. are in any case not detectable, while a large fraction of the material which causes blackening of the filter when sampling urban air is found in the smallest size ranges.

Particles of potassium iodide may also be collected by direct sedimentation on the surface of a membrane filter either dry or moistened with palladium chloride solution. Since the rate of deposition is normally very low, approximately equivalent to 0·1 l./min. on a 20 mm. diameter circle for particles of 7–8 μm. diameter with a settling velocity of 0·3 m./min., this is not usually a convenient method, but it does provide a direct method of estimating the settling velocity of the particles generated by any dispersing device.

Determination of the particle settling velocity

A 3·5 % solution of potassium iodide in 80 % ethyl alcohol was sprayed from a spinning disk atomizer into a room 3 m. × 5 m. × 3 m. high. The room was ventilated with approximately 200 $m.^3$/hr. of fresh air. After spraying had been in progress for 5 min., samples were taken at 3 l./min. through membrane filters at two positions in the room for the next 11 min. while at the same time two filters were exposed for settling. The number of particles collected on the two suction samples were estimated to be 6700 and 9150 respectively. The numbers collected on the settle samples were 282 and 295. The area of the 20 mm. circle counted is π cm.^{2}. Hence settling velocity (s) is given by

$$s = \frac{282+295}{2\pi} \times \frac{2\times 3000}{6700+9150} \text{ cm./min.}$$

$$= \frac{557}{2\pi} \times \frac{60}{15{,}850} \text{ m./min.}$$

$$= 0{\cdot}35 \text{ m./min.}$$

After the end of the 16th min. spraying was discontinued and samples taken at 3 l./min. at three positions in the room over the periods 19–20th, 25–27th, 30–34th and 37th–43rd min. The numbers of particles obtained in these samples are shown graphically in Fig. 2, from which the estimated die-away rate is 10·3/hr. Since the room has a volume of 45 m.^{3} the ventilation of 200 m.^{3}/hr. is equivalent to an air change rate of 4·5/hr. The extent by which the die-away rate exceeds this figure can be attributed to particle settling, i.e. a rate equivalent to 5·8 air changes/hr. Since the room was 3 m. high this corresponds to a settling rate (s) of 3 × 5·8 m./hr. or 0·29 m./min.

The difference between this value and that of 0·35 m./min. estimated from the ratio of the volumetric concentration of particles to the rate of settling onto a horizontal surface is certainly within the experimental errors of this determination. Together the observations confirm that the potassium iodide particles produced had the desired settling rate of approximately 0·3 m./min. The room used for this experiment was one of those forming the burns unit in which a systematic study of particle transport from room to room has been carried out using the particle tracer method described (Hambraeus & Sanderson, 1972).

Discoloration of floors by settled particles

Under some circumstances particles of potassium iodide which have settled on floors or other surfaces may oxidize and give rise to yellow iodine staining. This is not troublesome unless there is an unusually large amount of material deposited in any one place, as may happen from settling of large particles or droplets in the immediate vicinity of an imperfectly baffled humidifier or air-driven spray. The minimum density of deposit which we have found to produce a just perceptible stain on white- or light-coloured polyvinyl floor tile when left undisturbed is about 3 mg./m.^{2}. The use of cleaning agents containing hypochlorite or other powerful oxidizing agents is, of course, liable to accentuate this effect.

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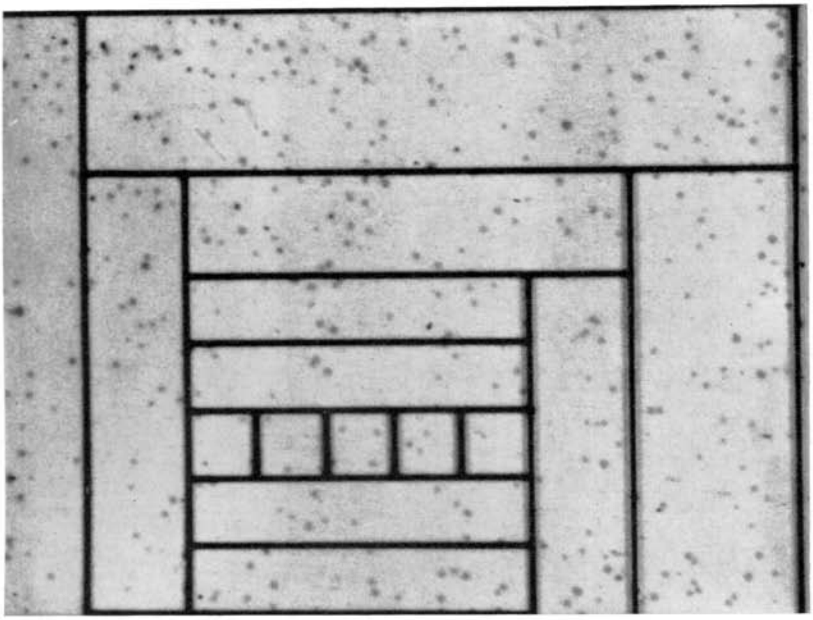
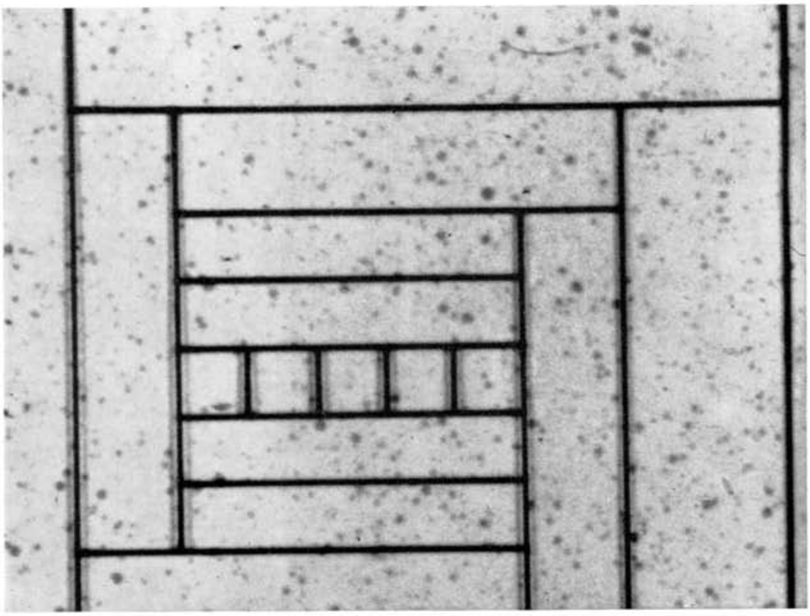
EXPLANATION OF PLATE

(*a*) Spots obtained on developing potassium iodide particles of nearly uniform size (*ca*. 8 μm.) produced by spraying a 3·5 % solution in 80 % ethyl alcohol from a spinning disk atomizer. The small squares in the centre of the counting grid shown over the developed filter are 0·8 × 0·8 mm. and each enclosure 0·2 % of the 20 mm. diameter sampling area on the 25 mm. diameter filter. The shadows alongside some of the grid lines arise from the combination of oblique two-sided illumination and imperfect contact between the graticule and the surface of the filter. (*b*) Similar to (*a*) except that the particles were generated by a baffled air spray, a simple scent spray head, using a 25 % aqueous solution of potassium iodide and an air pressure of 1·2 kg./cm.^{2}. There is a wide variation in the size of the particles produced.

Plate 1



(*a*)



(*b*)

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