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Impaction onto a Glass Slide or Agar versus Impingement into a Liquid for the Collection and Recovery of Airborne Microorganisms

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To study impaction versus impingement for the collection and recovery of viable airborne microorganisms, three new bioaerosol samplers have been designed and built. They differ from each other by the medium onto which the bioaerosol particles are collected (glass, agar, and liquid) but have the same inlet and collection geometries and the same sampling flow rate. The bioaerosol concentrations recorded by three different collection techniques have been compared with each other: impaction onto a glass slide, impaction onto an agar medium, and impingement into a liquid. It was found that the particle collection efficiency of agar slide impaction depends on the concentration of agar in the collection medium and on the sampling time, when samples are collected on a nonmoving agar slide. Impingement into a liquid showed anomalous behavior with respect to the sampling flow rate. Optimal sampling conditions in which all three new samplers exhibit the same overall sampling efficiency for nonbiological particles have been established. Inlet and collection efficiencies of about 100% have been achieved for all three devices at a sampling flow rate of 10 liters/min. The new agar slide impactor and the new impinger were then used to study the biological factors affecting the overall sampling efficiency. Laboratory experiments on the total recovery of a typical environmental microorganism, *Pseudomonas fluorescens* ATCC 13525, showed that both sampling methods, impaction and impingement, provided essentially the same total recovery when relatively nonstressed microorganisms were sampled under optimal sampling conditions. Comparison tests of the newly developed bioaerosol samplers with those commercially available showed that the incorporation of our research findings into the design of the new samplers yields better performance data than data from currently available samplers.

Naturally occurring airborne microorganisms consist mostly of bacteria, fungi, and viruses (34). Knowledge of their sources, concentrations, and biological activity is of great importance because of their potential health effects (11). Currently, there are no direct-reading instruments that indicate the presence and concentration of microorganisms. The collection (but not analysis) of bioaerosol particles is based on the same principles as those for nonbiological aerosols (10, 38). A fundamental requirement for microbial sampling is that the collected samples be undamaged and representative of the ambient environment (12, 22).

Available aerosol sampling techniques fall into several categories. The most important ones are gravitational sedimentation, inertial impaction, centrifugation, filtering, and electrical or thermal precipitation (28). Gravitational sedimentation is the principle of collection in settling plates which are frequently employed to collect microorganisms (50). However, the gravitational settling of particles is highly particle size dependent and strongly affected by air motion in the surrounding environment. Data obtained from the analysis of deposits on settling plates can, therefore, be used only to identify species present in the indoor air environment and not to quantify them (7, 38).

The term impactor encompasses a wide range of aerosol sampling instruments which have been used extensively to collect airborne microorganisms. Rotating impactors such as the Rotorod sampler (Ted Brown Associates, Los Altos Hills, Calif.) and the Rotoslide sampler (Oak Ridge Reproduction Service, Oak Ridge, Tenn.) collect particles larger than 15 μm and are commonly used for sampling outdoor pollen (39). These samplers function by sweeping a collection surface (such as a rod or a slide) through the air. The collection efficiency of such a rotating impactor is defined as the fraction of particles impacted on the collection surface from the volume of air swept by this surface. Since the air surrounding the impaction surface has no boundaries, this volume of air is difficult to define.

Suction-activated impactors usually collect microorganisms over a wide range of particle sizes. In such samplers, ambient aerosol is drawn through an inlet and impacts onto a soft agar or solid glass surface or into a liquid. The specific name for each impactor depends on what aspect of the device is emphasized. The following terms are currently used.

(i) Cascade impactor. The term cascade impactor emphasizes the fact that there are two or more stages, with each successive stage impacting particles of successively smaller size. For example, in the viable Andersen samplers (Graseby Andersen Inc., Atlanta, Ga.), each stage has 200 or 400 circular holes through which the bioaerosol particles impact onto an agar surface.

(ii) Slit sampler (e.g., Burkard Manufacturing Co. Ltd., Hertfordshire, United Kingdom; Barramundi Corp., Homosassa Springs, Fla.; New Brunswick Scientific Co., Edison, N.J.; Casella London Ltd., Bedford, United Kingdom; Lanzoni, Bologna, Italy). The term slit sampler emphasizes that the shape of the impaction nozzle is rectangular, not round. In such a device, the collection surface may be moved under the

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slit to spread the collected bioaerosol particles over a larger surface or to get a time trace of the bioaerosol particles present near the sampler.

(iii) Sieve-type sampler (e.g., Spiral System Instruments, Bethesda, Md.). The term sieve emphasizes that the collection stage consists of many holes, like a sieve. Such a device may consist of only one stage. The Andersen cascade impactors are also sieve-type samplers.

(iv) Impinger (e.g., Ace Glass Inc., Vineland, N.J.; Mine Safety Appliances Co., Pittsburgh, Pa.; Daco Products Co., Montclair, N.J.; A. W. Dixon Co., London, United Kingdom). Impingement is an alternate name for impaction and is commonly understood to denote impaction into a liquid rather than onto an agar surface or a glass slide.

In all impactors, the inertia of the bioaerosol particles is the primary cause for their removal from the airstream. While the airstream is deflected sideways, bioaerosol particles with sufficient inertia are impacted onto a soft or solid surface or into a liquid. In a centrifugal sampler (e.g., Biotest Diagnostics Corp., Fairfield, N.J.), the collection of bioaerosol particles is also due to their inertia. In the Biotest centrifugal sampler, ambient air is drawn into the sampler by means of a rotating impeller blade and is set into rotation. The centrifugal force on particles in the rotating airstream causes impaction of particles with sufficient inertia onto an agar-coated plastic strip. The collection efficiency of all of these suction-activated impactors is defined as the fraction of bioaerosol particles collected from the air drawn through the sampler.

Filtration devices can collect particles with almost 100% efficiency down to small particle sizes of about 0.1 μm (10), which is important when airborne viruses are being assessed. Furthermore, filtration is an easy-to-use method for sampling microorganisms in heavily contaminated environments (14) because the sampled organisms may be spread over a large filter area so that the collection medium does not overload in too short a time. However, filter samplers can cause the captured cells to become dehydrated, thus impairing their biological activity and making detection difficult (13, 38).

Particle precipitation from an airstream by an externally applied force, such as an electrical force on charged particles (25) or a thermal force in an aerosol flow with a thermal gradient perpendicular to its flow (4), is widely used for the size measurement of nonbiological aerosols. Such methods warrant consideration for bioaerosol sampling but are currently not applied in commercially available bioaerosol samplers.

The most commonly used methods for microbial collection today are impaction into agar, impaction onto a glass slide, and impingement into a liquid (8, 9, 15, 22, 26, 59). Many of the questions concerning their efficiency and accuracy during assessment of concentration levels of viable microorganisms are addressed in this study.

A variety of methods is available for sample analysis: culturing, direct microscopy, bioassay, biochemical assay, and immunological assay (7, 13). The most commonly used method for the assessment of viable airborne microorganisms is the cultural assay. The sample is collected onto an agar surface which can be cultivated directly to quantify the viable microorganisms. In the presence of high microbial concentrations, a sample collected into a liquid (impingement) has an advantage over a sample collected onto agar (impaction) because it can be diluted to the required level and can also be analyzed by several different assays. However, microbial clusters may break up during liquid impingement, potentially resulting in a higher bioaerosol particle count during sampling.

The performances of several viable bioaerosol samplers have been compared in different laboratory and field settings (16, 20, 24, 43, 49, 53, 56, 59). Many of the results obtained by the different authors contradict each other. Analysis of the bioaerosol samplers used in these studies shows that the samplers differ from each other considerably in their physical and biological collection aspects. Furthermore, different methods were used for microbial analysis, and airborne microorganisms were sampled from a variety of air environments. From these studies, it is difficult to conclude which microbial assessment method provides the best recovery of viable airborne microorganisms.

The overall objective of this research was to study the recovery of airborne microorganisms after sampling with the same inlet and collection geometries onto all three principle collection media: impaction onto a solid plate for particulate analysis, impaction onto an agar surface for cultural analysis, and impingement into a liquid for cultural analysis. Three new bioaerosol samplers were designed and built so that they differ only in the medium (glass, agar, or liquid) onto which the bioaerosol particles are collected. They are operated with the same flow rate through the same inlet and collection units. Analyses of the performances of these three different collection techniques have been conducted.

Analysis of the total recovery of airborne microorganisms. The total recovery of microorganisms obtained with a viable bioaerosol sampler is the product of the viability of airborne microorganisms in the environment and the overall sampling efficiency of the measurement device used. Microbial viability is usually understood as the organism's ability to multiply when provided with optimal conditions for growth (44, 47). The viability of airborne microorganisms is composed of three biological factors: microbial viability at their source, survival of organisms during their aerosolization, and survival of airborne microorganisms during their transport in the atmosphere. In the cultural assay, the viability data may vary depending on the medium used to culture microorganisms. The term survival is used here to mean the maintenance of viability under adverse circumstances. Microbial cells can become airborne through many mechanisms and then may remain viable for a long or short time, depending on various factors, such as pH level, amount of food available, action of metallic ions, oxygen activity, heating, or freezing (48). Microbial particle aerosolization as well as particulate transport in air environments can impose stress on the microorganisms, even to the point of death. Extensive information about different indoor microorganisms and their sources, sizes, and concentrations was summarized by Owen et al. (40).

The overall microbial sampling efficiency is the product of a number of physical and biological factors. The physical factors include particle aspiration, particle transmission through the sampling line, and collection or removal of the remaining particles from the air-stream. The biological factors include microbial survival during sampling and colony growth after sampling.

Particle aspiration (aerosol sampling from the ambient air into an inlet face) may lead to a loss or gain of particles due to changes in airflow speed and direction leading up to the inlet (54). The ratio of particle concentration at the face of the inlet to that in the undisturbed environment is defined as the aspiration efficiency. The larger the aerosol particles, the greater the over- or underestimation of the aerosol concentration is likely to be. Also, if there is ambient airflow and the sampler's inlet flow direction does not coincide with the ambient airflow direction, the measured aerosol concentration may be significantly different from that in the ambient air environment. The significance of this effect for bioaerosol sampling has recently been shown by Grinshpun et al. (17, 18).

Transmission efficiency is defined as the ratio of the aerosol concentration at the exit of the sampling line to that aspired at the inlet. It is a short distance in most viable samplers from the inlet to the sampling surface. However, some of them have a relatively long sampling line with bends and capillaries, for example, the AGI-30 impinger (Ace Glass Inc.). Aerosol particles, traveling such a route, can be lost due to a number of physical mechanisms within the sampler (6, 17, 21). Most of the particle loss occurs immediately after the inlet face is passed, but many of the particles may also be lost on the remaining interior surfaces of the sampler.

Once the particles are sampled through the inlet, preferably all of them are removed onto a collection surface. The collection efficiency is always a concern in bioaerosol sampling (15, 22, 37, 52, 57). Generally, the efficiency of a sampler in collecting a particle of a given size is related to the air velocity in the impaction nozzle. Too low a velocity in the inlet may result in failure to collect the particles of interest as they may never hit the collection surface; too high a velocity results in a high shear force which may cause serious damage to the bacteria, thus decreasing their viable recovery. The minimum velocity which allows collection of a particle of a given size can be decreased by decreasing the size of the impaction nozzle (29, 55).

The survival of viable microbial cells during sampling depends on the sampling method used and the fragility of the microorganisms sampled. The bacteria and fungi may be mechanically injured by high sampling flow rates in impactors and impingers and may not survive to grow as a viable culture (32). Long sampling times may also decrease the viability of the collected bacteria (8). Changes in cellular water content due to desiccation may add considerable stress to the collected bacteria (31). Exposure to oxygen toxicity is another possible factor affecting bacterial viability (51).

Postgate (45) has found that, as a result of various stresses, some bacteria lose the ability to multiply but remain functional otherwise. When appropriate growth conditions occur, these stressed bacteria may return to a culturable stage (47). Therefore, biological factors (including colony growth after air sampling and analysis of colonies) can strongly affect the precision of the microbial assessment. Marthi and coauthors (30, 31) have recently shown that the addition to the cultivation media of different materials (such as betaine, pyruvic acid, peptone, and catalase) can dramatically enhance the recovery of viable cells.

In addition to growth medium selection, the method and procedure of colony analysis are important. The analysis of viable microorganisms after their sampling requires a minimum number of colonies to obtain results with acceptable precision. Conversely, the colony surface density on an agar plate must not reach a level that will allow colonies to run together (14) since they must be counted while isolated. Decrease in colony counts may occur by the overgrowth of slow-growing cells by faster-growing colonies or by antibiotics which can kill neighboring cells or prevent their growth. For Andersen samplers (Graseby Andersen, Inc.), statistical probability tables are available to correct for the simultaneous impaction of two or more bacteria from the same impaction nozzle in a 200- or 400-hole impaction stage (27). Therefore, any method used to investigate the viable fraction of bioaerosols must have a carefully developed analytical procedure.

As seen from the above, total or near total recovery of airborne microorganisms through viable bioaerosol sampling is a complex process. For this reason, improvement of existing samplers and the development of new ones, as well as the interpretation of data, should be performed by analyzing each physical and biological component affecting the total recovery of viable microorganisms.

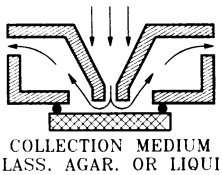

FIG. 1. Schematic diagram of three newly developed bioaerosol samplers.

MATERIALS AND METHODS

New bioaerosol samplers. Three new bioaerosol samplers have been developed for comparison of the three major bioaerosol collection techniques: impaction onto a glass slide, impaction into agar, and impingement into a liquid. These samplers differ from each other by the media on which the bioaerosol particles are collected, but they have the same inlet and collection geometries and the same sampling flow rate. Therefore, the physical components of the overall sampling efficiency are expected to be the same for all three new bioaerosol samplers. Differences in the total recovery of viable bioaerosols (if they exist) are, therefore, caused by biological sampling factors, as described in the previous section.

Identical inlet units were designed and built for all three new bioaerosol samplers, as seen in Fig. 1. All three inlet units consist of a narrow 0.2-mm-wide slot with a 60° tapered inlet. This slot width provides efficient particle collection at comparatively low particle velocity, thus facilitating the collection of microbial samples with minimal injuries. The slot length of 13.3 mm was chosen to essentially cover the entire width of the agar slide used for impaction (20.0 mm). It was found that the slot length should not exceed 14.2 mm to provide particle deposition within the agar slide surface. One or more slides may be moved under the slot to spread the bioaerosol particles over a wider area or to obtain a time-resolved analysis of airborne microorganisms near the sampler.

Previous work carried out by Willeke and Haberman (58) showed that the jet-to-plate distance has a strong effect on the particle collection efficiency. The jet-to-plate distance is defined as the distance from the exit plane of the impaction nozzle to the top of the surface onto which the particles impact. It was found that the measured impaction efficiencies are close to the theoretically predicted ones if the ratio of the impactor's slot width to the jet-to-plate distance is 0.5 to 4. As the jet-to-plate distance increases above seven slot widths, the cutoff size and the measure of spread of the impaction curve increase (i.e., under ideal conditions, all particles above the cutoff size are collected and all below this size remain airborne), but when the collection plate is too far from the impaction nozzle, the aerosol leaving the nozzle spreads and particle cutoff occurs over a range of particle sizes. Therefore, in all three newly developed bioaerosol samplers, the inlet unit is sealed to the collection unit, maintaining a jet-to-plate distance of 1.1 ± 0.1 mm. The aspirated air is extracted from the inlet unit, as shown in Fig. 1.

The aspiration and transmission efficiencies of the inlet stage were calculated as functions of the particle characteristics (such as aerodynamic size and density), inlet characteristics (such as size, geometry and orientation, and sampling flow rate), and the ambient conditions (such as wind velocity and turbulence). The equations listed in the studies by Brockmann

(6) and Grinshpun et al. (17) were employed. It was found that the sampling bias for the aspiration and transmission of 0.5- to 3.0-μm particles was less than 5% when sampling was at flow rates of 1 to 30 liters/min. However, this bias may increase when sampling is done in outdoor environments with relatively high wind velocities. For this reason, the inlet stage was designed in such a way that a special unit can be added later to reduce the inlet sampling bias in outdoor environments.

The collection unit of the glass slide impactor is a standard microscopic glass slide used for microbial cell collection. As the collection efficiency of a glass slide impactor depends on the physical properties of its collection surface (41), most measurements were performed with a double-sided sticky tape attached to the glass slide. The tape was then covered with a thin layer of petroleum jelly to prevent particle bounce from the hard glass surface.

In the agar slide impactor, the microorganisms are impacted onto an agar medium which can be moved under the inlet slot intermittently or continuously. Nunc slides (model 177372; Nunc Inc., Naperville, Ill.) were positioned inside the sampler for bioaerosol particle collection. These slides contain a chamber for an agar slide with a surface area of 20 by 42 mm^2. In preparation for the experiment, the slides were first cleaned and sterilized, using a Multy-Ray lamp with a germicidal tube which provides UV rays at 238 nm (Fisher Scientific, Pittsburgh, Pa.). The slides were then filled with 9.5 ml of tryptic soy agar (TSA) or R2A (both from Difco Laboratories, Detroit, Mich.). The prepared slides were stored at 5°C less than 4 h before sampling. They were kept capped to prevent drying of the agar surface.

The impinger is made of two parts, a long metal inlet (80 mm) and a Plexiglas beaker-like vessel sealed to the inlet by an O-ring. The Plexiglas vessel contains buffer for bioaerosol particle collection. In preparation for the experiment, the impinger was decontaminated by dipping it in boiling water for 10 min. Then, it was filled with 20 ml of sterile pH 7.0 sodium phosphate buffer at a molarity of 0.1 M (Na_2HPO_4 plus NaH_2PO_4). The same amount of phosphate buffer was used to wash the impinger after sampling.

All three new bioaerosol samplers were operated at various flow rates starting from 2 liters/min, which is typical for personal samplers, up to 12 liters/min. Bacterial collection efficiency was found to be nearly 100% at 12 liters/min, and further increase of the flow rate did not improve the collection efficiency.

Commercial samplers. Three widely used and commercially available bioaerosol samplers have been used for comparison with the related newly developed samplers. Two of the commercial samplers used for comparison are multiorifice cascade impactors: the Andersen VI-Stage Viable Particle Sizing Sampler, which is generally accepted as the standard instrument for viable bioaerosol particles (35), and the Andersen II-Stage Viable Particle Sizing Sampler (Graseby Andersen). The third was the Ace All-Glass Impinger (AGI-30; Ace Glass).

The Andersen VI-Stage Cascade Impactor operates at a sampling flow rate of 28.3 liters/min. Each stage of this sampler contains 400 orifices with diameters ranging from 1.81 mm in the first stage to 0.25 mm in the sixth stage. The corresponding cutoff sizes for the six stages are 7.0, 4.7, 3.3, 2.1, 1.1, and 0.65 μm (2). The collection plates for the sampler were prepared by pouring 45 ml of TSA aseptically into sterile plastic petri dishes (diameter, 100 mm; height, 15 mm).

The Andersen II-Stage Cascade Impactor also operates at a sampling flow rate of 28.3 liters/min. Each stage of this device contains 200 tapered orifices with diameters of 1.5 mm in the first stage and 0.4 mm in the second stage. The cutoff sizes are 8.0 μm for the first stage and 0.95 μm for the second stage (2). Each of the two petri dishes for this sampler contained 20 ml of TSA.

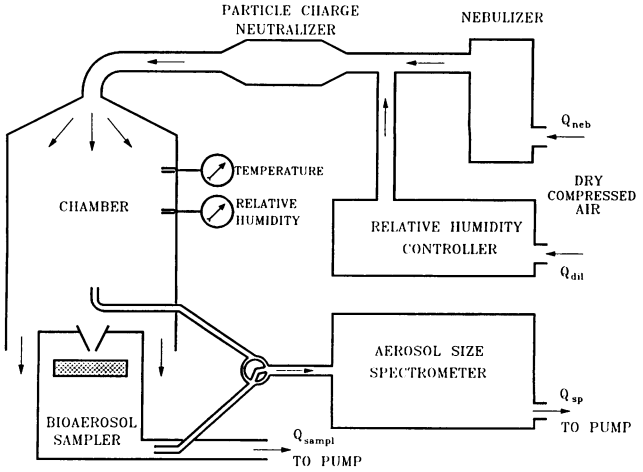

FIG. 2. Test system for the evaluation of bioaerosol sampler performance.

The AGI-30 impinger was operated at a flow rate of 12.5 liters/min. In this device, the aerosol is aspirated horizontally, redirected downward into a long vertical tube, and finally impinged into a liquid 30 mm from the bottom of a glass vessel. The reported cutoff size of this impinger is 0.3 μm (24). The impinger was filled with 20 ml of sterile phosphate buffer for microbial cell collection. As with the newly developed impinger, the same amount of phosphate buffer at pH 7.0 was used to wash the AGI-30 impinger after each test.

Test system. A new test system for the performance evaluation of bioaerosol samplers has recently been developed in our laboratory and has been described in detail by Thompson et al. (52). This system has been modified for the present study and used for the calibration of the three newly developed samplers, their laboratory evaluation, and their comparison with the commercially available samplers. The test system can be used to determine the overall sampling efficiency and the total recovery of any bioaerosol sampler. Its main features are described below and are schematically represented in Fig. 2.

As shown in Fig. 2, the system aerosolized the test microorganisms by means of a nebulizer. A Collison three-jet nebulizer (BGI Inc., Waltham, Mass.) was selected for dispersal of bacteria suspended in distilled water because it is composed of glass and stainless steel and, therefore, can be sterilized. Another important consideration in making this choice was that it has a fairly large reservoir of approximately 70 ml. This is important, as the aerosol concentration from a nebulizer will increase with time because the larger droplets return to the reservoir after partial evaporation and removal with the effluent flow of the liquid part of the bacterium-carrying and bacterium-free droplets (33). The aerosol size spectrometer measurements of the effluent bioaerosol concentration showed stable aerosol generation by the Collison three-jet nebulizer. The metal part of the nebulizer was grounded and the device was operated at a low air flow rate, Q_{neb} = 2 liters/min, to minimize bacterial stress during nebulization.

While this nebulizer has a stable aerosol generation rate, its output consists of water droplets, only a few of which contain bacteria but some of which may, generally, contain more than one bacterium. To minimize the presence of water droplets

carrying more than one single microorganism in each droplet, the bacterial concentration in the liquid was kept very low. To check whether the desired result was achieved, the aerosol size spectrometer was used to measure the particle size distribution after the droplets were removed by the addition of particle-free dilution air at a flow rate of Q_{dil} = 40 liters/min. It was found that only single bacterial cells and smaller droplet residues were registered by the instrument.

The aerosol was then diluted with prefiltered air which was either dry or humid, depending on the desired relative humidity. To provide enough time to dry the droplets of a few micrometers in size, the two air flows were combined and mixed in a grounded copper tube, 830 mm long and 26.7 mm in diameter. A 10-mCi Kr-85 particle charge neutralizer (model 3012; TSI Inc., St. Paul, Minn.) was built into the system to prevent electrostatic particle removal to the system surfaces. Exposure to a low-level radiation source was desirable because electrostatic particle removal could become significant after droplets containing bacteria shrink to a smaller size due to evaporation. The bioaerosol sampler then collected the microorganisms from a chamber onto a glass or agar surface or into an impinger.

The bioaerosol flow entered the chamber at a flow rate of 42 liters/min. The test sampler drew bioaerosols from this chamber (135 mm high; 115 mm in diameter), as shown in Fig. 2. The temperature and relative humidity inside the chamber were measured by a thermohygrometer (model DHTD; Fisher Scientific) and were adjusted by the relative humidity controller. As the bioaerosol flow rate through the test system exceeded the bioaerosol sampler's flow rate, the remaining bioaerosol from the chamber was discarded into a class II biological safety cabinet (SterilchemGARD; Baker Co., Inc., Sanford, Maine), which housed the entire system.

The number concentration of each aerosol size fraction and the resulting particle size distributions upstream and downstream from the bioaerosol sampler were measured with an aerosol size spectrometer (model LAS-X; Particle Measuring Systems, Inc., Boulder, Colo.). The peak of the particle size distribution, measured with the LAS-X, corresponded to the expected size of aerosolized microorganisms. The aerosol size spectrometer measured the particle size distribution from 0.09 to 3 μm. This instrument used identical sampling probes to sample upstream and downstream from the bioaerosol sampler. The flow rate in the test system was essentially undisturbed because the sampling flow rate of the aerosol size spectrometer was only 0.06 liters/min.

Calibration of new samplers. Particle separation from an airstream is usually characterized by the particle's aerodynamic diameter, which is defined as the size of a unit-density (1 g/cm^3) sphere that has the same gravitational settling velocity as the particle in question (4, 23, 46). Most bacterial cells are nonspherical particles with equivalent aerodynamic diameters ranging from fractions of a micrometer to several micrometers. In order to calibrate the three new samplers as to their collection efficiency, monodisperse polystyrene latex (PSL) particles (Dow Chemical Co., Indianapolis, Ind.) were used. Their sizes, d_p, ranged from 0.22 to 1.09 μm. This is the size range over which the new samplers were designed to have their cutoff sizes for efficient particle collection, depending on the flow rate used. The PSL particles were dispersed by the same nebulizer, and all aerosol measurements were made in the test system shown in Fig. 2.

Of principal interest in this study were the new samplers' collection efficiencies under a variety of sampling conditions. As the overall sampling efficiency consists of the inlet sampling efficiency and the collection efficiency, the calibration of the samplers was performed in two steps. First, the collection surface was removed from the sampler, and the sampled upstream and downstream aerosol concentrations (C_0 and C_s, respectively) were measured. The inlet sampling efficiency, E_s, was thus determined as:

$$E_s = \frac{C_s}{C_0} \tag{1}$$

Second, the collection surface was placed back into the sampler, and the aerosol concentration downstream from the sampler, C_{out}, was measured. The collection efficiency, E_c, was thus determined as:

$$E_c = \frac{C_s - C_{out}}{C_s} \tag{2}$$

Test microorganism. The microorganism used for testing was *Pseudomonas fluorescens* ATCC 13525 (American Type Culture Collection Inc., Rockville, Md.). This bacterium was selected because it is known to be a common constituent of bioaerosols (36). *P. fluorescens* was stored in deep agar slants of TSA at a temperature of 5°C. By using this source, the transfer of cells was made, and growth from single-colony isolates was used in the assays. Cells were streaked onto TSA plates and incubated at 25°C for 16 h. These cells were then harvested in 50 ml of sterile pH 7.0 sodium phosphate buffer, 0.1 M. Using a centrifuge (model Marathon 6K; Fisher Scientific) at 2,860 × *g*, the suspension was washed twice with phosphate buffer and once in deionized water to remove the salts. To match the turbidity of the 0.5 standard of the McFarland nephelometer tubes (3), the washed suspension was then diluted with sterile deionized water. This suspension was further diluted to 1:200 and kept at 5°C until use.

Viability of microbial cells in suspension. To determine the viability of microorganisms in the suspension used for nebulization, a microbial sample of 0.08 ml was taken from the undiluted suspension with an inoculating loop (Fisher Scientific). It was placed onto a glass slide within an area of 100 mm^2 for direct microscopic counting. An identical sample was inoculated onto an agar slide with the same area for cultural analysis. To avoid overgrowth of microcolonies, this sample was taken from a diluted suspension. The cells on the glass slide were stained with Gram stain prior to counting. The number, $(N_{cell})_{susp}$, of the stained bacterial cells on the glass slide was counted by a bright-field phase-contrast microscope (Labophot-2; Nikon Corp., Tokyo, Japan), using ×400 magnification. The number, $(N_{col})_{susp}$, of microcolonies developed on the agar slide after 16 h of incubation at 25°C was counted with the same microscope, using ×100 magnification. The microbial cell viability, V, in the suspension was calculated as:

$$V = k \frac{(N_{col})_{susp}}{(N_{cell})_{susp}} \tag{3}$$

where k is the dilution coefficient. The measurement was usually repeated five times, and the average value was used as a representative value of the bacterial viability in the suspension. The coefficient of variation did not exceed 10%.

Microbial sampling and sample analysis. All samples were taken from a bioaerosol chamber which was operated at a relative humidity of 30% ± 2% and a temperature of 22 ± 1°C. The newly developed samplers were evaluated at sampling flow rates of 2 to 12 liters/min. When their performances were compared with those of the commercially available bioaerosol samplers, the sampling flow rate for all three new devices was

fixed at Q_{sampl} = 10 liters/min, while the commercial ones were operated at their standard flow rates (28.3 liters/min for both Andersen samplers and 12.5 liters/min for the AGI-30). The sampling time was set at 3 min for the new agar slide impactor and both Andersen samplers and at 10 min for the new glass slide impactor, the new impinger, and the AGI-30 impingers. Collection onto agar surfaces was limited to 3 min to prevent colony overcrowding, which may occur if the indicated samplers are operated longer.

The bacterial samples, which were collected onto the TSA slides of the new agar slide impactor and on plates of both Andersen samplers, were cultured directly. Prior to incubation of the agar slides, melted agar was added to cover the slide surfaces in order to decrease the desiccation effect of the sampling process. For both impingers, bacterial samples were collected into phosphate buffer from which pour plates were then prepared with TSA. These plates were incubated at 25°C for 48 h and counted with a Quebec Colony Counter (Fisher Scientific). The CFU recovered on the agar slides were counted as described above.

The total recovery, R, of the bioaerosol particles was calculated as the ratio of the number of colonies, $(N_{col})_{sampl}$, developed after microbial sampling and incubation to the total number of microorganisms, $(N_{part})_{sampl}$, counted in the sampled air volume by the aerosol size spectrometer.

$$R = \frac{(N_{col})_{sampl}}{(N_{part})_{sampl}} \quad (4)$$

The latter component was obtained by multiplying the bioaerosol particle concentration, C_0, measured with the aerosol size spectrometer upstream from the sampler by the volume of sampled air:

$$N_{part} = C_0 Q_{sampl} T_{sampl} \quad (5)$$

where Q_{sampl} is the sampler's flow rate and T_{sampl} is the sampling time.

RESULTS AND DISCUSSION

Inlet sampling efficiency. The inlet sampling efficiency of newly developed and commercially available bioaerosol samplers evaluated with the equations listed in references 6 and 17 was found to be 100% ± 5% for spherical aerosol particles smaller than 3 μm. Direct measurement of E_s carried out, first, with PSL particles of 0.22 to 1.09 μm and then with *P. fluorescens* confirmed this result. However, as indicated above, the inlet sampling efficiency may differ significantly from 100% if these samplers are used for the collection of larger bioaerosol particles (such as fungi or pollen).

Particle collection efficiency. As all three samplers employ the same impaction principle for particle collection, the first experiments were designed to find the maximum collection efficiency attainable with the new design. Figure 3 shows the measured collection efficiency dependence on the flow rate through the glass slide impactor. The coefficient of variation of the data presented in Fig. 3 did not exceed 10%. The glass slide was rendered sticky, as described above, in order to avoid particle bounce from the collection surface. As seen in Fig. 3, particles larger than 1 μm in aerodynamic diameter can be collected efficiently when the sampling flow rate is 4 liters/min or higher. This particle size range is typical for cutoff sizes of commercially available personal samplers. The new bioaerosol samplers were designed so that a variety of personal pumps can be used with these new samplers to collect microorganisms with an equivalent aerodynamic size greater than 1 μm. A number of personal pumps are available in industrial hygiene practice for attachment to the belt of a worker for "personal monitoring." They are quiet and reasonably light in weight and, thus, have an advantage over larger-flow-rate pumps that are noisy and necessitate use of an electrical outlet. To sample bioaerosol particles of smaller size (about 0.7 μm), a sampling flow rate of 7 liters/min or greater should be set up to achieve efficient particle collection. For this purpose, personal pumps operating at a Q_{sampl} of up to 8 liters/min, such as the recently introduced Genesis Air Sampler (model gn-8p; Ametek Inc., Largo, Fla.), are appropriate. The efficient collection of 0.5-μm (or smaller) particles can be attained at a sampling flow rate of 10 liters/min or higher.

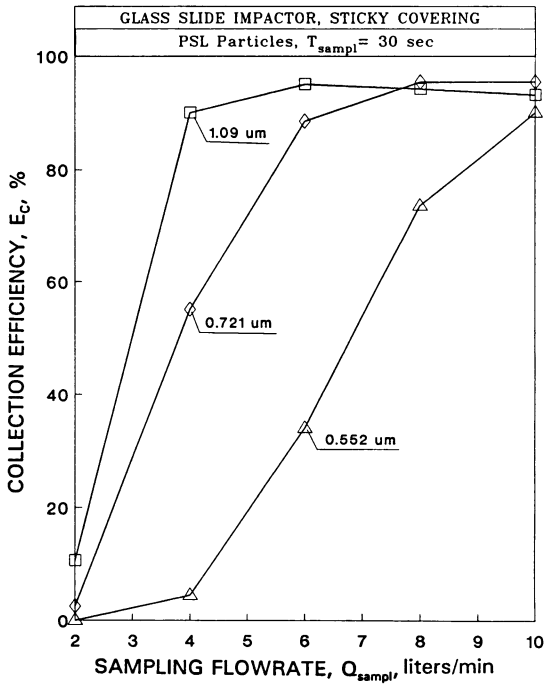

FIG. 3. Maximum collection efficiency attainable with the new glass slide impactor.

Figure 4 shows the particle-size-dependent collection efficiency for each of the three newly developed samplers at a sampling flow rate of Q_{sampl} = 10 liters/min. As seen in Fig. 4a, the particle collection efficiency of the glass slide impactor is

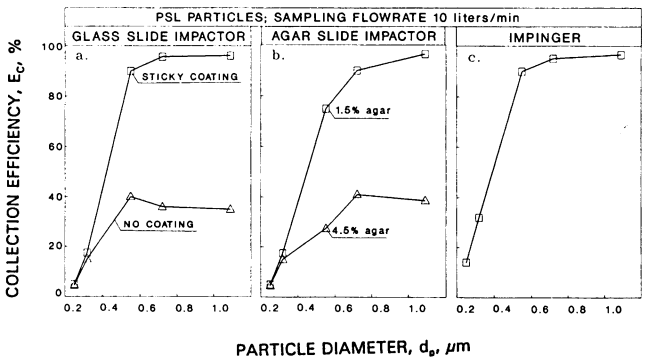

FIG. 4. Comparison of the collection efficiencies of the three newly developed bioaerosol samplers.

less than 40% when sampling is done onto the regular glass slide without its surface being coated. It can be increased to about 100% upon application of a special coating. However, coating of the glass slide makes it difficult to stain bacteria for direct microscopic counting. Sampling of microorganisms onto noncoated glass slides, which is the normal practice today, may therefore not collect all microorganisms and may, to some degree, be particle size dependent. The sampling time for both experiments was 30 s.

The particle collection efficiency of the agar slide impactor is shown in Fig. 4b. The sampling time was only 15 s for this experiment, as explained further below. As seen, the efficiency of PSL particle collection turned out to be very sensitive to the relative density of agar in the collection medium. Experiments on the variation of this parameter showed that the best collection characteristics of the agar slide impactor were reached when the concentration of agar in the TSA collection medium was about 1.5%. In this case, we were able to collect more than 90% of aerosol particles with diameters larger than 0.7 μm. However, the particle collection efficiency dropped to about 40% when the agar concentration in the collection medium increased to 4.5%. In this case, the agar surface apparently became nonsticky, and impacted particles rebounded similarly to those on the glass slide impactor without any coating.

Figure 4c demonstrates that, when aerosol particles are sampled into the impinger at 10 liters/min, the particle collection curve is similar to the optimal ones obtained for the glass and agar slide impactors. Therefore, use of the above-described optimal particle collection media allows us to achieve the same overall physical collection efficiency whether sampling onto a solid surface (impaction onto glass or agar slide) or into a liquid (impingement). Similarity of the curves shows that, in impingers, other physical removal mechanisms (such as particle diffusion from the bubbles in the liquid into the liquid) are of minor importance relative to removal by impaction.

Particle collection onto an agar slide was investigated not only for different agar concentrations but also for different sampling times. As mentioned above, upon sampling onto a more concentrated agar (4.5%), there is a significant decrease of the particle collection efficiency (Fig. 4b) most likely due to particle rebound from a relatively nonsticky surface. Increase in the sampling time results in drying of the agar surface, which, in turn, apparently leads to an increase of particle rebound. This, therefore, leads to a decrease in particle collection. Figure 5 demonstrates this effect when sampling at a 10-liters/min flow rate onto a nonmoving Nunc slide filled with 1.5% agar in TSA. For example, when particles of 1-μm size were sampled, an increase in sampling time from 15 to 180 s caused the collection efficiency to decrease from more than 90% to less than 60%. This means that, when sampling for bacteria, an extended sampling time could result in the underestimation of the microbial concentration level. However, in many practical situations, a short sampling time may not be representative of the environment.

The use of a moving agar slide instead of a stationary one increases the actual impaction field, which allows a significant increase in the sampling time for a given surface density of collected particles. Furthermore, the exposure time of a given area of the collection surface to the drying air jet is reduced significantly. It eliminates the sampling time effect. The two sampling situations, moving versus nonmoving (stationary) agar slides, were analyzed as shown in Fig. 5. The evaluation of the physical particle collection efficiency for a moving agar slide impactor was performed at a sampling time of 180 s. This sampling time was obtained at a constant slide motion speed of

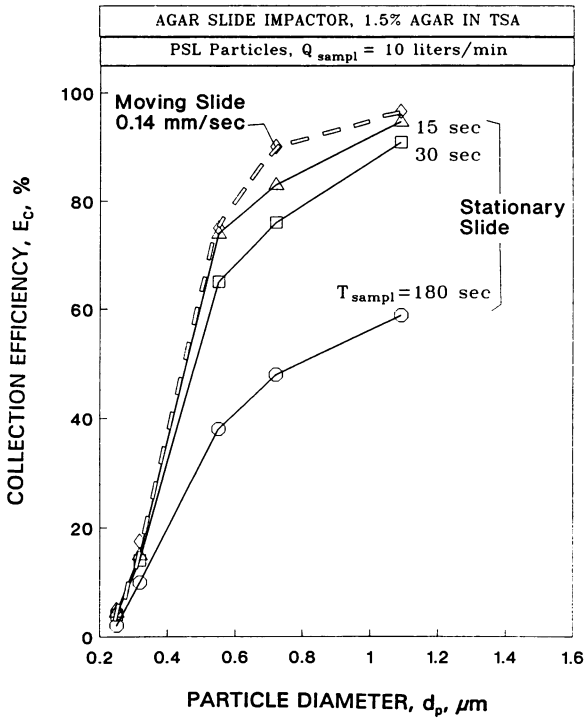

FIG. 5. Effect of sampling time on particle collection on agar.

0.14 mm/s when PSL particles were impacted onto the 25-mm-long collection field. Figure 5 shows that more than 95% of aerosol particles of 1 μm and larger were collected onto the moving agar slide, while less than 60% of these particles were collected onto the stationary agar slide during the same sampling time of 180 s.

Figure 6 demonstrates that the dependence of the particle collection efficiency on the sampling flow rate is similar for both new impactors; with the moving agar slide and with the sticky glass slide (the data presented in Fig. 6 were obtained with a coefficient of variation of 5 to 15%). However, the new impinger does not provide good particle collection at 6 to 8 liters/min. This effect turned out to be even more pronounced for larger particles. We interpret that this irregular behavior of the impinger collection efficiency curve is caused by particle interaction with the collection medium. Upon sampling at a relatively low flow rate of 2 liters/min, nearly 20% of the aerosol particles were impacted into the liquid layer under the inlet slot. At an increased sampling flow rate of 4 liters/min, the impinger's collection efficiency increased to about 50%, as seen in Fig. 6. Such values are theoretically expected (55). However, against expectations, further increases of the flow rate from 4 to 8 liters/min did not improve the particle collection efficiency. A possible explanation is that the liquid layer under the impinger's inlet slot was removed by the pressure created by the air jet, and aerosol particles were impacted directly onto the bottom of the collection vessel. That caused the rebound of the impacted PSL particles from the hard plastic surface. Therefore, some aerosol particles were resuspended in the airflow and escaped the impinger with the effluent airflow. At higher sampling flow rates of 10 and 12 liters/min, the inertia of the rebounded aerosol particles was, apparently, high enough for their secondary impaction into the surrounding liquid. Figure 6 demonstrates that the particle collection efficiency of the impinger at flow rates of 10 liters/min and

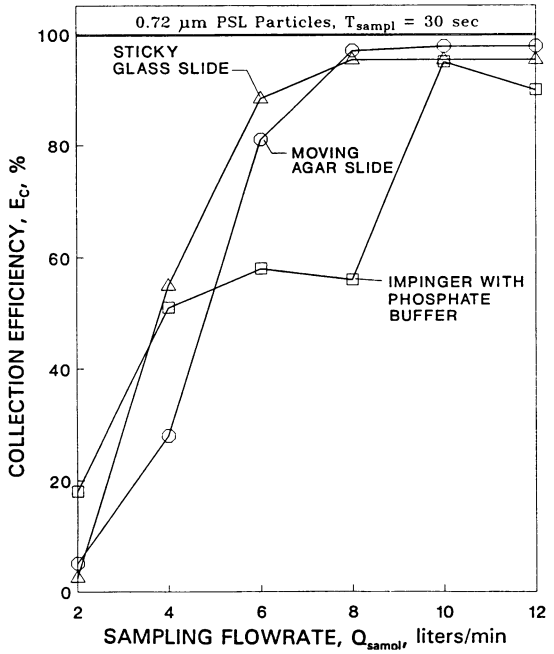

FIG. 6. Anomalous collection characteristic of liquid impinger.

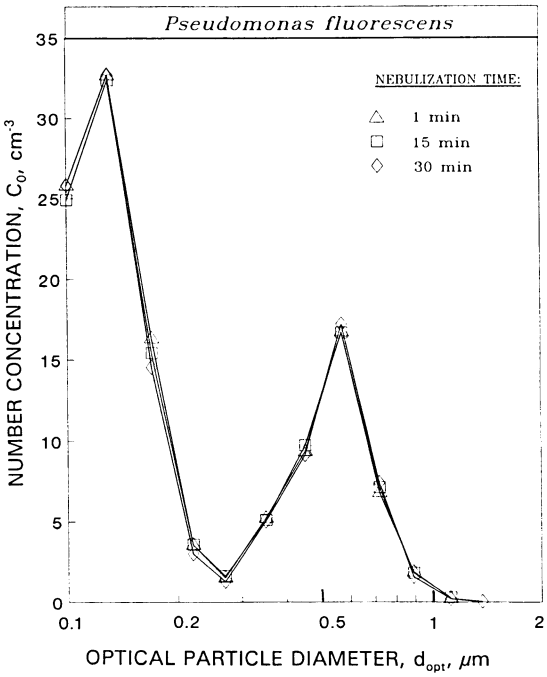

FIG. 7. Measured particle concentrations upstream from the sampler after various nebulization times.

higher was similar to those of the glass slide and agar slide impactors. More detailed information about these new findings will be presented by Grinshpun et al. (19).

Total recovery. Analysis of the total recovery of viable microorganisms showed that the overall sampling efficiency is composed of physical and biological factors. The establishment of optimal sampling conditions allowed us to eliminate the effect of the physical sampling efficiency and concentrate on the effect of biological sampling factors on the total recovery of viable microorganisms. The newly developed agar slide impactor and impinger were used to study impaction onto a solid surface versus impingement into a liquid for the total recovery of *P. fluorescens.*

Figure 7 demonstrates that the airborne particle concentration was very stable during the aerosolization of the washed suspension of *P. fluorescens*. The smaller particle size mode (0.1 to 0.3 μm) was created by droplet residues, which consisted of salts from the phosphate buffer, growth medium, and, possibly, small portions of damaged bacteria. The larger particle size mode was created mostly by single bacterial cells. A few doublets were also registered on the right tail of this mode. Breed et al. (5) have listed the size of *P. fluorescens* to be 0.3 to 0.5 μm in diameter and 1.0 to 1.5 μm in length, while Palleroni (42) has given a somewhat larger size range of 0.7 to 0.8 μm in diameter and 1.5 to 3.0 μm in length. These differences could be caused by the use of various growth media, differing times of cell harvest (1), or dissimilarities in measurement methods and growth conditions. It is seen in Fig. 7 that our optical bacterial cell diameter, d_{opt}, as measured by the aerosol size spectrometer (an optical light-scattering instrument), ranged from 0.3 to 1.5 μm. The bacterial cell concentration achieved its maximum at the optical particle size of about 0.55 μm. In these experiments, nonisometric bacterial cells with different orientations in the airflow were measured with the optical aerosol size spectrometer. Therefore, variation in the orientation of elongated cells of the same size and shape in the laser beam of the measuring device may have resulted in a variation of the optical particle sizes recorded by the instrument.

The total recovery of *P. fluorescens* was determined for the two new viable samplers, the agar slide impactor and the impinger, at sampling flow rates ranging from 2 to 12 liters/min (Fig. 8). Upon sampling with the agar slide impactor, the total recovery increased from almost 0% at a sampling flow rate of 2 liters/min up to about 25% at 10 liters/min (Fig. 8, left). The total recovery curve has a shape similar to the one obtained for the particle collection efficiency (Fig. 6). Figure 8 (left) also shows that the value of the total recovery can be essentially the same for various media, as demonstrated by the use of R2A

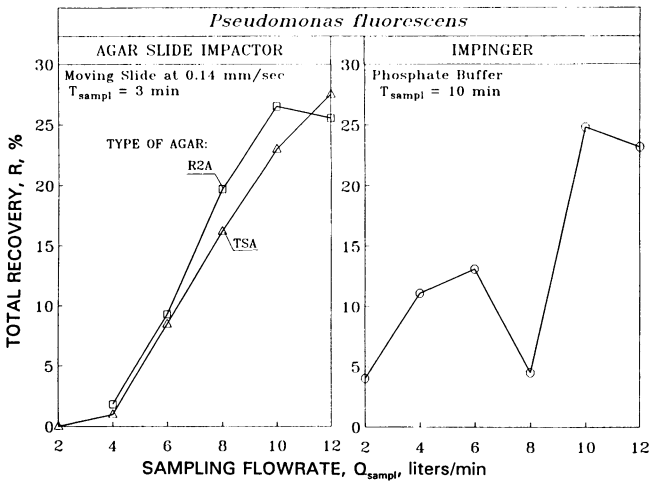

FIG. 8. Total recovery of *P. fluorescens* as a function of sampling flow rate.

TABLE 1. Laboratory comparison of the performance of the two newly developed versus three commercially available viable bioaerosol samplers

Bioaerosol sampler	Flow rate (liters/min)	Total % recovery (SD)[a]
New agar slide impactor	10.0	26.0 (4.2)
New impinger	10.0	24.2 (4.1)
Andersen II-Stage Viable Sampler	28.3	1.25 (0.14)
Andersen VI-Stage Viable Sampler	28.3	16.8 (1.3)[b], 21.1 (1.6)[c]
Ace All-Glass Impinger, AGI-30	12.5	9.85 (2.10)

[a] *P. fluorescens.*
[b] Before correction.
[c] After correction.

versus TSA. This result warrants further exploration, as it is important for the practical use of the agar slide impactor in indoor air sampling where several different agar media may be used to collect multicomponent bioaerosols, including bacteria and fungi.

Figure 8 (right) demonstrates the anomalous behavior of the total bacterial recovery when sampling is into an impinger at a flow rate of 8 liters/min. This is a result of the irregular behavior of the impinger's particle collection efficiency (shown in Fig. 6 and discussed above). Also, it is seen in Fig. 8 (right) that the total recovery of *P. fluorescens* at low sampling flow rates of 2 to 4 liters/min was higher for the impinger than for the agar slide impactor. At an optimal sampling flow rate of 10 to 12 liters/min, at which we have a physical sampling efficiency of close to 100% for both the agar slide impactor and the impinger, the total recoveries of microorganisms were similar for both bioaerosol samplers. Thus, we can conclude that, when comparatively nonstressed microorganisms are sampled at optimal sampling conditions, the biological factors involved in the impaction and impingement processes are similar.

Comparison of newly developed and commercially available viable bioaerosol samplers. The two newly developed viable samplers (the agar slide impactor and the impinger) and three commercially available viable samplers were tested under the same ambient conditions for the total recovery of the airborne bacterium *P. fluorescens*, using the above-described test system (Fig. 2). The results obtained are shown in Table 1. As expected, the lowest total recovery of 1.25% was found with the second stage of the Andersen II-Stage Viable Sampler (at a cutoff size of 0.95 μm). The highest total recovery values of 26.0 and 24.2% were achieved by the newly developed agar slide impactor and impinger, respectively. The sixth stage of the Andersen VI-Stage Viable Sampler recovered 16.8% of aerosolized bacteria. This value is increased to 21.1% after the positive hole method (27) is applied to correct the experimental data but still remains notably lower than the total recovery value obtained with the new agar slide impactor. The All-Glass Impinger (AGI-30) exhibited a low total recovery value of 9.85%, although its cutoff size is about 0.3 μm. The data on the relative performance of the two Andersen impactors and the AGI-30 obtained in our comparative tests are in good agreement with the results of the intercomparison of commercial bioaerosol samplers published by Nevalainen et al. (38) and Jensen et al. (24). On the other hand, some of the data of Lembke et al. (26) and Zimmerman et al. (59) demonstrate a higher level of microorganisms from their impinger assays than from the impactor. The latter result represents the situation when the breakup of large microbial clumps in the impinger is predominant.

Experiments on microbial viability in the suspension demonstrated that about 25 to 30% of the bacterial cells suspended in the deionized water during the comparison tests were culturable. Hence, the value of the microbial viability before sampling (indicated as V in equation 3) was only slightly higher than the value of the total recovery of airborne bacterial cells after their sampling with both newly developed viable bioaerosol samplers (indicated as R in equation 4). This demonstrates that almost all viable bacterial cells survived during sampling with the new viable bioaerosol samplers (on the agar slide and in the liquid) and remained culturable after sampling. In addition, it shows that the loss of viable particles during aerosolization and transport in our test system was negligible. This was confirmed by multiple assays. Therefore, we conclude that impaction onto an agar surface may provide the same overall sampling efficiency as impingement into a liquid, if the sampler's inlet and collection design (including properties of the collection media as well as the sampling flow rate and time) are optimized.

Further experiments employing environmentally stressed and aggregated microbial cells are planned and will provide important information about the applicability limits of these microbial sampling methods.

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