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Naval Biomedical Research Laboratory, Programmed Environment, Aerosol Facility

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Mathematical considerations of the behavior of aerosolized particles in a rotating drum are presented, and the rotating drum as an aerosol-holding device is compared with a stirred settling chamber. The basic overall design elements of a facility employing eight rotating drums are presented. This facility provides an environment in which temperature can be maintained within 0.5 F (0.25 C) of any set point over a range of 50 to 120 F (10 to 49 C); concomitantly the relative humidity within any selected drum may be controlled in a nominal range of 0 to 90%. Some of the major technical aspects of operating this facility are also presented, including handling of air support systems, aerosol production, animal exposure, aerosol monitoring, and sampling.

Early studies on the ability of selected microorganisms to survive when suspended as an aerosol were conducted in stationary chambers, usually with provisions to maintain internal stirred settling during the period of observation. When longer observation times were required, taller test chambers had to be constructed since the mean aerosol-holding time is directly proportional to the effective height of the holding chamber. Unfortunately, this approach is costly and, from practical considerations, self-limiting.

An alternate concept for long-term aerosol holding led to the development of the slowly rotating drum (5). Utilizing this rotating-chamber concept, the duration of observation periods could be extended by nearly two orders of magnitude, and the mean aerosol-holding time was shown to be independent of the rotating chamber diameter. Thus, the size of the rotating holding chamber is dictated by aerosol sampling requirements and not by a desired aerosol holding time.

THEORETICAL CONSIDERATIONS OF AEROSOL-HOLDING CHAMBERS

Early studies on biological aerosol stability were limited in observation time by the physical loss which takes place within the aerosol-holding chamber. A test aerosol usually was held in a simple closed chamber and maintained by stirred settling. In an aerosol-holding chamber 1 m in height, a particle of unit density 1 μm in diameter would have a physical half-life ($T_{1/2}$) of 6.4 hr. Since $T_{1/2}$ is proportional to H/a^2, where H is the effective height of the test chamber and a is the radius of the aerosolized particle, a particle 4 μm in diameter would have a $T_{1/2}$ of 6.4/16 hr or 24 min. Several approaches may be considered to increase $T_{1/2}$: the height of the test chamber increased from 1 to 10 m will result in a $T_{1/2}$ of 4 hr for a particle 4 μm in diameter. Although such a height increase is not unreasonable, construction costs can become excessive when this approach is used; therefore, other possible holding techniques should be considered.

One of the most successful alternate techniques is the rotating drum. With this technique, an increase in experimental aerosol-holding time of over 20-fold can be effected, as compared with a stirred settling aerosol chamber 1 m in height. It is of value to quantitatively compare these two aerosol-holding techniques.

Consider first a vertical, cylindrical tank of height H and cross sectional area A. The rate of loss of a test aerosol, assuming stirred settling, can be expressed as follows:

$$\begin{aligned} d(C)/C &= -yAdt/AH \\ &= -(mgdt/6\pi a\eta)(A/AH) \\ &= -mgdt/6\pi a\eta H \end{aligned} \quad (1)$$

where H = height of chamber, a = radius of particle, A = cross sectional area of chamber, C = aerosol particulate concentration, v = Stokes velocity, m = mass of particle ($4/3\pi a^3 d$), d = density of particle, g = gravitational constant, η = viscosity of air, and where the term "$mg/6\pi a\eta$" defines the Stokes velocity of free fall

for a particle of radius a and mass m. It is assumed in this equation that the entire physical loss takes place by settling onto the floor area. After integration, we have

$$C_t = C_{t=0}e^{-\frac{mg}{6\pi a\eta}\frac{t}{H}} \quad (2)$$

It is evident from equation 2 that an increase in effective aerosol-holding time can be achieved only by increasing the height H of the test chamber.

Now consider a slowly rotating drum as an aerosol test chamber. Imagine a particle within a vertical plane passing through the axis of rotation. Gravity results in a velocity of free fall, $v = mg/6\pi a\eta$, which, in the postulated initial position, results in a vector of free fall towards the axis of rotation. As the drum slowly rotates, the particle continues to fall away from the reference plane until this plane has rotated 180°. Thus, the particle moves toward the outer wall of the rotating drum and back to the initial reference point. Upon completion of a full drum rotation, the particle will have completed a full circular orbit of radius r_a . The plane of the circular orbit will be tangential to the selected reference plane. (For simplicity, the centrifugal effects of rotation are being neglected.)

Since the velocity of free fall multiplied by the time required to complete one orbit determines the circumference of one orbit, the orbital circumference is equal to the product of the velocity of free fall of the particle (centimeters per second) times the reciprocal of the rate of rotation of the drum expressed as (revolutions per second)$^{-1}$. Thus

$$2\pi r_a = 2ga^2(d_1 - d_2)/9\eta\theta \quad (3)$$

where θ = revolutions per second.

Now let us consider the rotating drum in a more realistic fashion. The combined effect of rotation and gravity is to produce a circular orbital motion of each suspended particle within the drum. Centrifugal forces generated by the rotation of the drum produce a slow, outward radial drift of particles. Now imagine that each such particle system is replaced at the center of rotation of each orbital system by a particle of the same mass. We now have to consider the centrifugal field of force acting on each of these equivalent systems.

Analytically, this becomes $f = m\omega^2R$, where ω = angular velocity of the rotating drum in radians per second and R = radius of rotation of a selected particle system.

Two models, each resulting in physical loss of the contained aerosol within the rotating drum, can be considered: (i) tranquil movement of the contained aerosol towards the outer wall, resulting in an expanding axial annulus which becomes cleared of the test aerosol; (ii) gentle stirred mixing within the drum, resulting in a uniform aerosol distribution within the rotating drum, coupled with a slow peripheral physical loss due to impingement on the outer wall.

Experimentally it has been observed that, even after several days of undisturbed slow rotation of the drum, the aerosol distribution remains uniform from the axial region to the outer boundary. Thus it is clearly demonstrated that gentle stirring, coupled with centrifugal drift to the outer boundary, should be considered as a mathematical model of physical loss. This can be expressed mathematically as

$$dC/Cdt = -(m\omega^2R/6\pi a\eta)(2\pi RW/\pi R^2W) \quad (4)$$

where R = radius of the rotating drum, W = axial width, and ω = angular rotation in radians per second. Upon integration, one obtains

$$C_t = C_{t=0}e^{-\frac{m\omega^2t}{3\pi a\eta}} \quad (5)$$

Let us consider some of the parameters in a quantitative fashion. Examine the circular orbit executed by a particle 6 μm in diameter in a drum rotating at 2 rev/min. We have

$$2\pi r_0 = [2ga^3(d_1 - d_2)/9\eta\theta]\cdot(60 \text{ sec}/1 \text{ min}); \quad r_0 = 0.51 \text{ cm} \quad (6)$$

where θ = revolutions per minute, g = 980 cm/sec^2, $\eta = 181 \times 10^{-6}$ poise (dyne-sec/cm^2), and $a = 3 \times 10^{-4}$ cm (6 μm in diameter), with $(d_1 - d_2)$ nominally assumed to be 1.0.

Now consider the equation defining physical loss in a slowly rotating drum:

$$C_t = C_{t=0}e^{-\frac{m\omega^2t}{3\pi a\eta}} \quad (7)$$

where $m = 4/3\pi a^3(d_1 - d_2)$.

Now define

$$K_{rd} = m\omega^2/3\pi a\eta = [4a^2(d_1 - d_2)/9\eta]\omega^2 \quad (8)$$

One notes that K_{rd} is a function of the squared product of two variables, i.e., $a^2\omega^2$, and is independent of the radius R of the drum. It is evident that this simplification is only true when r_0 is much smaller than R. In practice, R is usually measured in meters and r_0 is in millimeters or centimeters. The rated physical loss of a particle increases as the square of its diameter; hence, a particle 6 μm in diameter will have an expected physical loss of 36 times that of a particle 1 μm in diameter.

Table 1 provides a comparison of the theoretical physical loss in a slowly rotating drum with the expected physical loss in a 1-m-high vertical tank, assuming stirred settling.

Experimentally, 2 rev/min has been successfully used with an observed $T_{1/2}$ as long as 60 hr for particles of nominally 1.0 μm in diameter. This is almost a 20-fold increase in duration of holding time in comparison with that attainable in a 1-m-high chamber.

It is of interest to note that a rotating drum system is extremely sensitive to structural vibration. The first unit was unfortunately mounted in a manner which resulted in structural vibration. The second major installation provided an improvement in this respect. The current installation further isolated the rotating drums from both building and drive motor vibration, thus providing a closer agreement between the derived theoretical physical half-life and the observed data.

Further illustrative comparison of a rotating drum with a stationary stirred settling chamber is of interest. This comparison can be obtained by again using the mathematical expression for K_T and K_{RD}, which simplifies to the following:

$$\omega = (g/2H)^{1/2} \quad (9)$$

Now, with θ arbitrarily set at 2 rev/min ($\omega = \pi/15$ radians per sec), we have

$$H = g/2\omega^2 = 980/2(\pi/15)^2 = 11{,}300 \text{ cm or } 113 \text{ m} \quad (10)$$

Experimentally, values roughly one-fifth of those computed by using equation 8 have been observed with a drum 2 m in diameter. This would place a drum rotating at 2 rev/min as equivalent to a 23-m-high stirred settling chamber. It would be quite a problem to provide effective stirred settling conditions in a tank 23 m in height.

TABLE 1. *Expected physical half-life as a function of particle diameter, assuming stirred settling in a 1-m-high tank (T) versus a drum rotating at 2 rev/min (RD)*

Particle diameter (μm)	$T_{1/2}$, RD (hr)	$T_{1/2}$, T (hr)
1	710	6.4
1.5	315	2.85
2	177	1.6
4	44	0.40[a]
6	20	0.18[b]

[a] Twenty-four minutes.
[b] Eleven minutes.

PRACTICAL CONSIDERATIONS OF THE EIGHT-DRUM FACILITY

General description. Earlier work on survival of microorganisms in aerosols (1, 6) emphasized the need for a larger facility in which replicate aerosols created from a single test culture preparation could be simultaneously subjected to different environmental conditions. Such a facility, consisting of eight stainless-steel, rotating chambers ("drums") of nominally 1,000 liters each, was designed and constructed as four sets of twin-drum units (Fig. 1). The eight drums rotating at 2 rev/min are housed in a temperature-controlled room. Each twin set of drums is supported by one rotating shaft. Manipulations to the system are carried out from the laboratory work hood and room apposing the drum room or from the adjacent machinery room. The environmental parameters of the eight-drum facility are controlled by three independent systems: the air-circulating system, the heating-cooling system, and the process air system.

Air-circulating system. Six fans maintain the air circulating through absolute filters between the drum holding room and the work hoods, thus reducing any accidental aerosol to minimal levels within minutes. In addition, two exhaust fans evacuate air from the drum room through absolute filters. This airflow rate is maintained at a level sufficient to create a negative pressure of 0.5 inch (1.27 cm) of water in the drum room and work hoods. Further, the work hoods are negative to the pressure maintained in the laboratory work room, which in turn is negative to the rest of the laboratory structure.

Pressure in the drums, maintained by the process air system at 2 inches (5.08 cm) of water, protects the validity of the experimental work by preventing inward leakage of unwanted environmental constituents; concomitantly, an operator is protected from exposure to possible leaks in the system by the intervening barrier of air at negative pressure in the hoods and drum room.

Heating-cooling system. Any desired temperature can be maintained within a range of 50 to 120 F (10 to 49 C) with a fluctuation about a set point of 0.5 F (0.25 C). This is achieved by heat transfer between the heating-cooling coils and the circulating air.

The work hoods are insulated to minimize heat loss. An additional external heat source in the machinery room precludes any condensate forming in the lines at that point.

Process air system. Figure 2 schematically illustrates the air handling technique for each of the eight rotating drums. A regulated, pressurized flow of filtered dry air is split into two flows. One

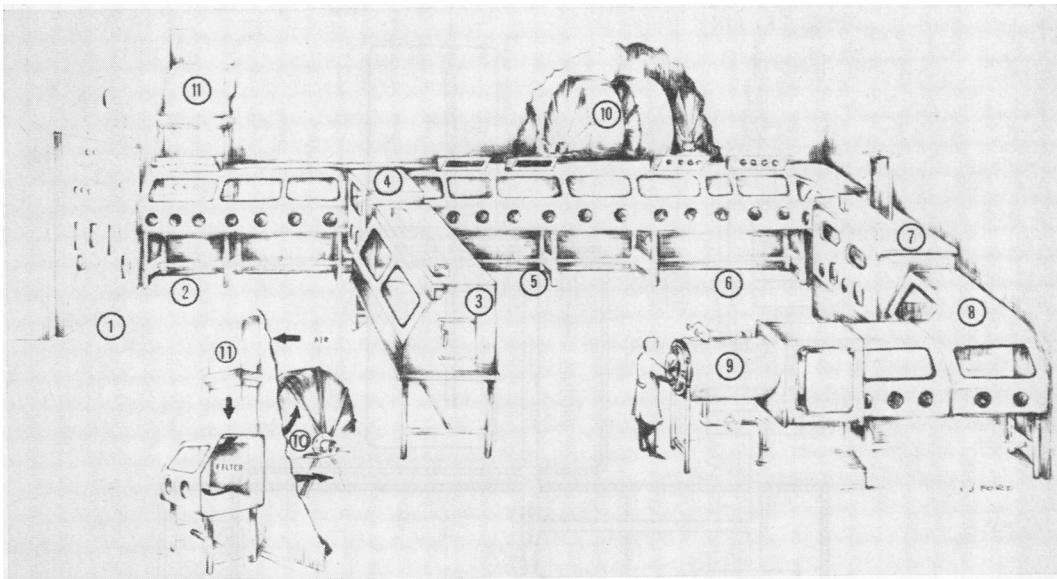

FIG. 1. *General layout of the Naval Biomedical Research Laboratory Programmed Environment, eight-drum aerosol facility. (1) Control panel with automatic controls and monitoring devices. The machinery room is in apposition. (2) Fill hood. From this location all drums are filled with aerosol. Guillotine door at first partition confines this area of greatest hazard. All hoods against the drum room wall and including the incubator are insulated to minimize heat loss. (3) Class II work hood. When hood is converted to class III, end opening permits introduction of materials. (4) Air lock. Guillotine door on either side protects the pressure gradient within the hoods. (5) Hood space for animal exposure. (6) Position of sample ports for all eight drums. (7) Two-level, elevator-operated incubator. (8) Room temperature hood space for enumeration. (9) Double-ended autoclave. (10) Several of the eight drums mounted in apposing room (see inset). (11) One of the eight return air ducts by which air is recycled between hood and drum room (see inset).*

portion is bubbled through a tank of temperature-controlled distilled water, providing a downstream airflow at relative humidity (RH) of 90%. The remaining dry air goes through a manually controlled restriction so that the downstream dry and wet airflows can be balanced to equal pressures as they enter the mixing valves. This pressure at the mixing valve is adjusted to nominally 1 lb/inch^2. Dry air from 0 to 20 parts is mixed with wet air from 20 to 0 parts, thus providing humidity control in increment steps of approximately 5%. For example, air consisting of 3 parts of wet air and 17 parts of dry air results in a downstream flow of 13.5% RH. The total processed air flow of the eight drums is 20 ft^3/min.

During sampling a replacement airflow is automatically supplied from the processed air for sampling rates of as much as 2 ft^3/min. This replacement air, at the same RH and temperature as the air contained in the drum, enters via the four symmetrically placed tubes rotating with the drum and projecting some two-thirds of the radial distance into the drum.

During a drum air-washing period before a new experimental run, some 2 ft^3/min of processed air is drawn through each of the rotating drums. Air washing normally is continued for a minimum of 10 hr or for some 30 air exchanges. An excess of 0.5 ft^3 per min per drum is bypassed through an absolute filter and flows into the sampling hood as indicated in Fig. 2. The pressure drop through the filter establishes a minimal positive drum pressure of 0.5 inch of water during air washing, thus eliminating any possibility of unprocessed air entering the drum.

Surveillance. The control panel to the left of the spray hood provides for constant monitoring of all systems and environmental parameters.

Decontamination. If desired, cleaning and decontamination of the drum room can be achieved by using a steam line installed in the drum room. Provision is made for addition of formaldehyde or other vapor decontaminants.

Work flow. Because of the potential hazard inherent in aerosol studies, the facility is designed and equipped so that all materials are brought in through a class II work hood and passed through a double-door airlock into the class III work hoods which are equipped for all required aerosol procedures (2). Plates may be incubated in a two-

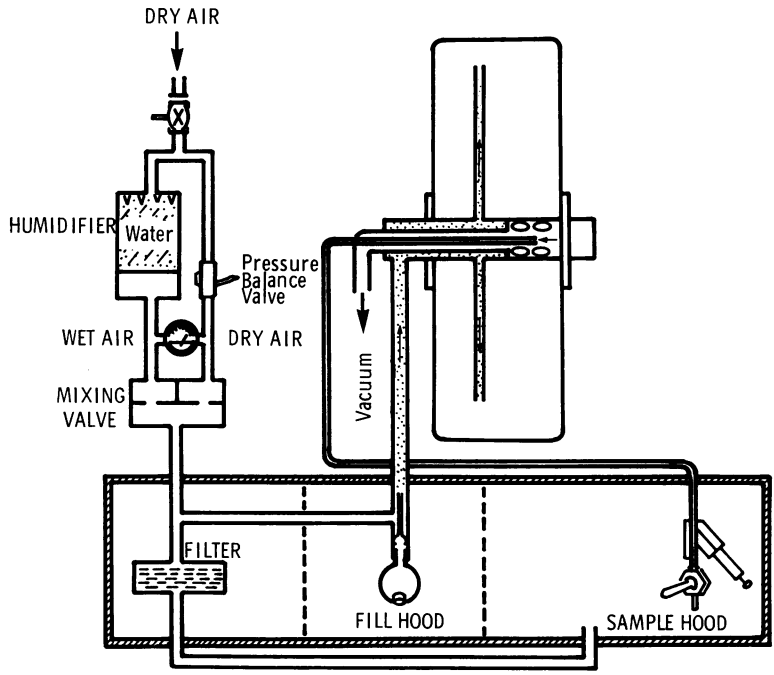

FIG. 2. *Process air system.*

level, elevator-operated incubator and then passed to the other side for enumeration. All material is transported by this route to the double-ended autoclave where it is processed before removal from the system.

Aerosol production and drum filling. Beginning 5 min or more before the aerosol filling period and continuing for a minimum of 5 min after filling, the drum rotation is cycled: a 30-sec rotation period alternating with a 30-sec stationary period. This expedites a rapid and uniform aerosol distribution within the drum.

Material to be atomized is adjusted so that a desired aerosol concentration can be achieved by a nominal 1-min atomization period. The aerosol from a modified all-glass Wells refluxing nebulizer is introduced into the processed airflow which in turn is introduced axially into the drum (Fig. 2). After atomization, air is drawn past the atomizer into the fill line for a 30-sec period to clear the upstream drum-fill lines of generated aerosol. Atomization introduces a slight increase in drum moisture, but, by limiting the aerosolization period to 1 min, this effect is less than 1% RH within the mixed drum volume. The humidity in the process-filling line is raised no more than 10% for a period not exceeding 1 min. (The larger axial exhaust tube indicated in Fig. 2 is used to minimize the pressure drop within the test drum during aerosol filling or subsequent air washing.) Airflow through the drum is then shut off, and the entire 2.5 ft^3 of processed airflow per min bypasses through the filter and into the sampling hood; the resulting pressure drop creates a drum pressure of some 3 inches (7.62 cm) of water.

Measurement of temperature and humidity. Before introducing a test aerosol into a drum for experimental observation, the holding temperature and RH within each drum are determined. These two environmental parameters are of major significance in controlling microbial survival in the airborne state. The measurement of temperature can be accurately and easily obtained by a number of sensing devices: thermocouple, resistance bulb, and standard capillary mercury thermometer. The measurement of humidity in the temperature range of 50 to 120 F (10 to 49 C) can be measured reasonably accurately with a calibrated "in-line" wet bulb/dry bulb technique.

However, a more sensitive and accurate low-range humidity determination can be made by using a commercially available dewpoint sensor (Cambridge, model 992). This unit provides an expanded scale in the RH region below 20%. In our hands, an "in-line" wet bulb/dry bulb technique provides a reading of approximately 10% RH (standard psychrometric charts) when the true air humidity is less than 1%. An "in-line" wet bulb/dry bulb unit can be calibrated against a dewpoint sensor to provide meaningful data from

nominally 0 to 95% RH. It must be noted, however, that the change in wet bulb temperature is small for changes in RH below 20% due to the limitations imposed by in-line measurements. The use of a wet sock with no liquid reservoir is of value in slightly reducing the measured wet bulb readings, but the heat received from the surrounding walls still remains as a controlling factor in raising indicated wet bulb readings over those obtained by using a standard outdoor sling psychrometer. An additional disadvantage of the wet bulb technique is the requirement for a relatively large sampled air volume for satisfactory humidity readings. By contrast, an accurate dewpoint reading can be obtained from a few liters of sampled air, thus making it feasible to measure drum humidities during the course of an experiment, if such is required.

Aerosol monitoring. An electro-optical aerosol monitoring system is provided to allow for measurement of physical loss, thus any accidental error in air handling can be rapidly noted. In addition, the use of the syringe air-sampling procedure requires a quantitative measure of the aerosol concentration at the point of syringe sampling to insure the accuracy of this sampling procedure. This is especially critical when an aerosol sample of 1 cm^3 is required.

Light scatter was selected as the most simple and convenient technique for aerosol monitoring (Fig. 3). A test aerosol introduced through T1 passes through the diaphragm, D3, and exits through T3. The hole in the diaphragm D3 is focused by lens L2 as an enlarged image on the plane of diaphragm D4. The image of the hole in diaphragm D3, focused on the plane of diaphragm D4, is illustrated in the figure inset. The hole in diaphragm D4 is smaller than the image of the hole of diaphragm D3. Hence, the photomultiplier views the scattered light from the introduced test aerosol against an image of the hole of diaphragm D3. Since a hole is inherently clean, this optical design is not subject to electronic instability from continual usage. The electronic circuit design provides for an eight-decade range. In terms of a test aerosol, this encompasses light scatter from the molecular level up to visually dense smoke. The most sensitive range provides for a minimal indicated reading for test aerosols of nominally 1,000 particles greater than 0.5 μm in diameter, per liter of sampled air. A usable, stable reading is obtained for test aerosol concentrations of 10^4 such particles per liter. This level is equivalent to normal "clean" city air.

Sampling. Aerosol samples are withdrawn from the drum through a stationary tube which is projected roughly half-way down the supporting hollow axle (Fig. 2). Samples are taken usually every 15 min for the first 0.5 to 1 hr and then hourly to every other hour for an additional 8 to 12 hr. Viable assays define recovery as a function of aerosol-holding time.

To optimize the use of an eight-drum experimental aerosol facility invites an analysis of day-to-day operational requirements. Time-saving sampling techniques have been devised which reduce total operating manpower requirements to reasonable levels. For example, with the syringe aerosol-sample technique, which takes a sample directly from the aerosol to the nutrient agar surface for bacterial assay, a minimum of sample processing is involved as compared with the dilution, plating, and dallying required when a liquid impinger sample must be processed. Direct aero-

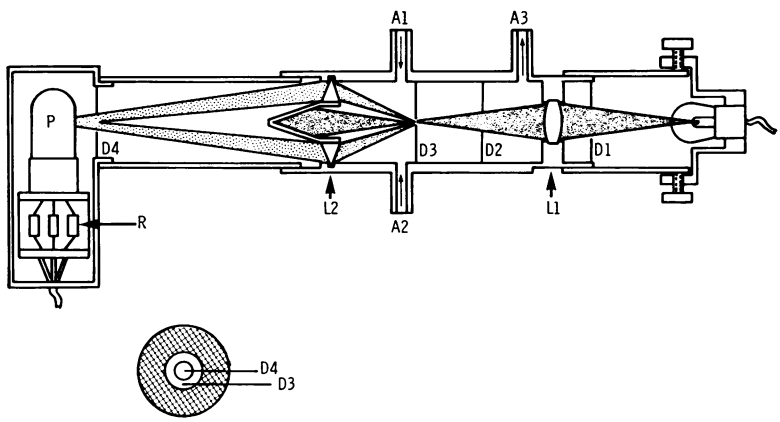

FIG. 3. *Goldberg, forward-angle, light-scatter, particulate monitor (patented). A1, sample air inlet; A2, filtered air inlet; A3, air exhaust to vacuum; D1, D2, D3, D4, a series of diaphragms; L1, lens; L2, lens with central light trap; P, scattered light sensor; R, resistor used in dynode circuit.*

sol-to-agar assays can be obtained by using a syringe of either 1-, 10-, or 100-cm^3 volume. Similarly, direct sampling onto agar plates from the drum via the slit sampler for 6 sec, 1 min, or 10 min will correspond to aerosol volumes of 1, 10, or 100 liters per plate, respectively, thus covering a concentration range from 1 to 100,000. The range of countable colony formation per standard petri plate (from nominally 10 to as high as 1,000 colonies per plate) extends the quantifiable range to 10^7. A maximal sampled volume of 100 liters is compatible with the drum capacity; however, at the end of an aerosol observation period, 10-min samples may be taken as desired.

When one observes the aerosol created from a Wells nebulizer, the physical loss within a slowly rotating drum is so small that it is practicable to extend sampling periods over 1 to 2 weeks, with tolerable physical losses. With normal sampling, the observed physical half-life of a test aerosol is in excess of 2 days; if sampling is held to a minimum, the observed half-life may be extended to in excess of several hundred hours.

When particles larger than 3 μm in diameter are sampled, the sampling line should be as short as possible to minimize wall losses. For test aerosols in the 1- to 2-μm diameter range, however, a sampling line [0.25 inch (0.64 cm) inner diameter] 20 to 30 ft in length can be used with minimal wall losses at sampling rates of 10 to 20 liters/min.

Nutrient plates for bacterial assays are introduced into the work hood and are usually equilibrated in temperature before receiving the aerosol sample.

Syringe aerosol-sampling technique. A test aerosol is drawn past the tip of the syringe. The physical concentration of the aerosol at this sample point is continually monitored by a forward-angle light-scatter device placed just downstream of the sampling position. The electro-optical reading is taken and recorded just before sampling.

For sampling, the plunger of the syringe is depressed and held in for at least 3 sec before it is slowly released to withdraw the sample volume. A slight pause is taken before removing the filled syringe to allow for pressure equilibration.

The tip of the syringe is inserted into a fixture on the slit-to-agar sampler (Fig. 4) which provides for a 10 liter/min annulus of clean filtered air of the same temperature and humidity as the sampled aerosol. The slit sampler lid is closed and pausing for a moment allows a steady airflow to be established; the syringe plunger is then slowly depressed and again held for a moment at the end of the stroke. The slit sampler vacuum is released, and the agar plate is covered and immediately placed at incubator temperature.

The use of a syringe for aerosol sampling is limited to test aerosols with particles of less than nominally 2 μm in diameter. As the particle diameter increases, syringe wall losses become a limiting factor and cause recovery to be skewed in an undesirable manner.

Slit sampling. Physical design parameters of slit sampling have been discussed in some detail previously (3). In summary, they include (i) desired sampling rate, (ii) suitable slit width and length, and (iii) slit-to-agar allowed clearance.

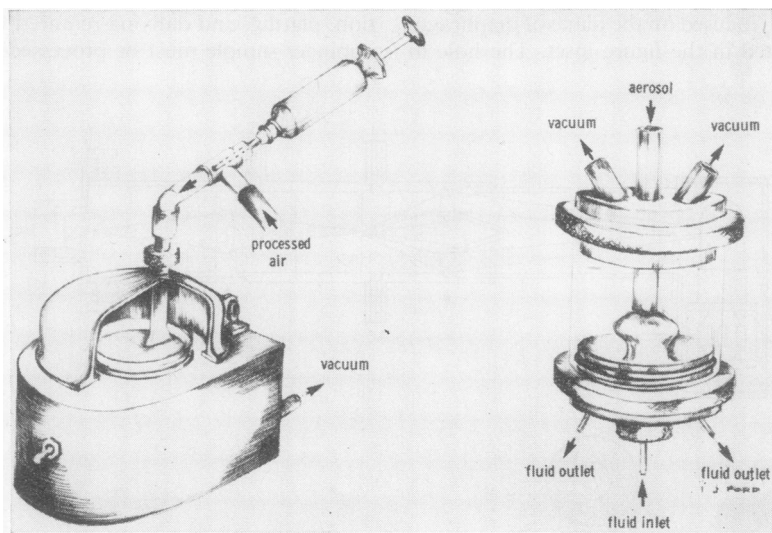

FIG. 4. *Sampling devices. (Left) Slit-to-agar sampler with aerosol sampling syringe in position to deposit sample for direct assay of viable organisms. (Right) Continuous impinger. The aerosol is continuously injected at the top and impinged upon collecting fluid which is injected at the bottom and may be recycled.*

When sampling directly from a nominal 1,000-liter chamber volume, a sampling rate must be used to minimize the effects of sampling itself upon the observed viable aerosol survival. A sampling rate of 10 liters/min was selected and was provided by a slit opening of 0.010 by 0.50 inch (0.025 by 1.27 cm). A careful balance must be obtained in impaction efficiency as a function of particle diameter to minimize viable impaction losses when sampling fragile rod-shaped microorganisms.

To minimize the accumulation of particulate material in the narrow slit exit, the interior of the tubing end should first be brought to a high polish before forming the slit. The slit is formed over an inserted piece of shim stock of the required thickness by flattening the end of the tubing so that a gradual taper results and provides an exit slit with the required dimension; the length of the slit is predetermined by the inner diameter of the tube. An 0.5- to 1.0-mm slit-to-agar clearance is necessary for quantitative particulate impingement onto the agar. Careful microscopic examination of the impaction zone under such a slit demonstrates a clear slit image impaction area, with little if any halo or splatter surrounding this deposit area. With solid media plates, precision flat-poured to provide the proper slit clearance, and a plate rotation speed of 60 or 120 rev/min, an even distribution of incubated colony count is obtained.

Impinger samplers. Two other sampling devices are used, primarily for viral aerosol sampling (Fig. 4): the capillary impinger operated at 12.5 liters/min and the Naval Biomedical Laboratory (NBL) continuous impinger (4).

The capillary impinger is normally operated with 10 to 20 ml of sampling fluid for periods up to 5 min. As with the slit sampler, the impaction parameters defined by a sonic airflow through a capillary can result in viable cell rupture with a reduction in expected colony or plaque formation. As the sampling rate is increased by increasing the capillary diameter, the probability of cell rupture decreases. Unfortunately, however, as capillary size is further increased, the efficiency of recovery as a function of particle size decreases for particles of a defined size or smaller. Hence, there is a maximum sampling rate to obtain optimal recovery of viable aerosolized cells of a given size size range. When the capillary impinger, with the tip of the impinger slightly above the resting liquid level, is used to collect aerosolized particulates emanating from a modified Wells nebulizer, a near-optimal impinger samping rate is 12.5 liters/min. The efficiency of viable recovery achieved at 12.5 liters/min compared with 6 liters/min was distinctly superior particularly when sampling fragile rod-shaped organisms, as opposed to spherical cocci. The effect noted can be directly attributed to a reduction in cell rupture with a 12.5 liters/min impinger rather than an increase in particulate impingement efficiency.

Correspondingly, observed viable recovery can be increased by reducing the impinger sampling rate through use of subsonic sampling velocity. In particular, a 6-liter/min "critical flow" capillary impinger operated by using a vacuum of 12 inches of Hg can provide a higher viable recovery per unit volume of sampled air because of the decreased cell rupture achieved by a subsonic airflow.

The NBL continuous impinger is available in

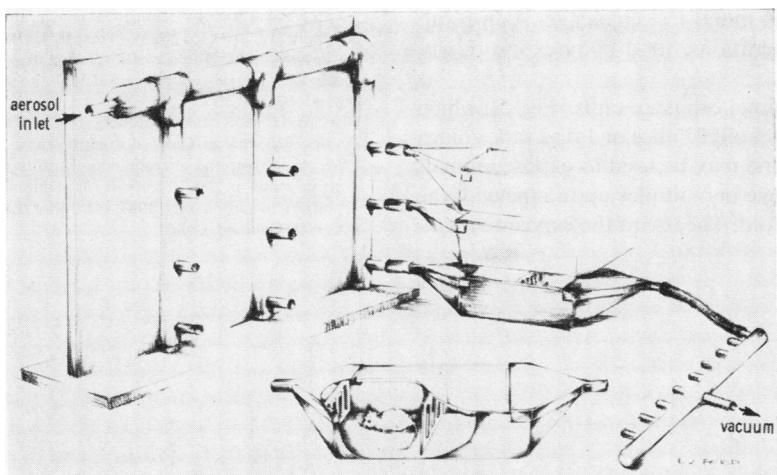

FIG. 5. *Apparatus for animal exposure. Individual cages (see inset), in any number up to nine, are attached to the exposure manifold and to the exhaust manifold containing restrictive orifices.*

two models: one has an aerosol sampling rate of 12.5 liters/min and the other model has a rate of 27 liters/min; both are normally operated with a liquid sampling flow rate of 1 cm^3/min. This sampling technique is most suitable for recovering residual viable content in the drum after a long-term aerosol-holding period. Some 50% of the residual particulate content of the drum can be recovered in less than 2 ml of final fluid volume, thus proving a capability of recovering one viable survivor from a population of 10^9 aerosolized particles. This sampler is especially valuable for viral aerosol assays.

Animal exposure. Two general approaches have been used for animal exposure to aerosols. In one, the aerosol exposure is restricted to the head of the test animal; with large animals, such as the monkey, the aerosol exposure may be even further restricted to the mouth and nose. The other approach utilizes total body exposure to the aerosol. This low-stress exposure method requires a minimum of physical restraint of the test animal and lends itself to more reproducible response.

If one allows total body exposure during an aerosol challenge, a secondary ingested dose will occur from the fur washing by the animal. The relative level of coat retention versus inhalation retention is strongly dependent upon particle size. Utilizing a modified refluxing Wells nebulizer, the particulates in the test aerosol have a nominal size range of 1 to 2 μm in diameter. For the albino mouse, the total coat collection is only 25% that of the inhaled dose. Of the inhaled dose, only nominally 10% is retained in the lung; the remaining nominal 90% is ingested. So the subsequent increase in ingested dose resulting from coat licking is quite nominal when compared with the dose results from the initial test challenge. As a result of these considerations, total body exposure was selected.

The small animal exposure units (Fig. 5), which will individually hold 12 mice or 1 or 2 rats, guinea pigs, or hamsters, may be used to expose animals to a test challenge in multiples up to nine with the exposure manifold. The use of the exposure manifold allows for 108 mice to be simultaneously exposed to a single test aerosol.

SUMMARY

The theoretical considerations which led to the construction of a multiple rotating-drum aerosol facility have been summarized. The techniques of air handling, drum aerosol filling, sampling, animal exposure, and physical monitoring have been considered, providing a summary of the physical aspects of this new aerobiological facility.

As a result of the outlined advantages, the rotating-drum holding chamber has been accepted as an International Standard for aerosol holding. Detailed drawings of the unit can be obtained by writing to the Naval Biomedical Research Laboratory, School of Public Health, University of California, Berkeley.

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