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A Slit Sampler for Collecting T-3 Bacteriophage and Venezuelan Equine Encephalomyelitis Virus

I. Studies with T-3 Bacteriophage

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Continuous sampling of air to detect pathogens is essential in the conduct of hazardous operations and in monitoring infectious disease wards, and could have significance in instances of massive air pollution. Sampling devices employing liquid collecting media for the impingement of air-borne microorganisms, although quite satisfactory for laboratory use in the sampling of air for short periods of time, do not meet the requirements for satisfactory sampling over long periods. Problems associated with liquid impingers are (a) evaporation of the collecting fluid, (b) the relatively small volume of air passing through the sampler limits these devices to sampling of relatively highly concentrated aerosols, and (c) the high air velocity is detrimental to the survival of certain organisms after 10 to 15 min of operation (Shipe, Tyler, and Chapman, 1959; Tyler, Shipe, and Painter, 1959). These disadvantages have been overcome in the sampling of bacterial aerosols by the use of slit samplers in which the organisms are impacted directly upon a solid collecting medium which also serves as a nutrient during incubation of the sample. Many studies have shown these samplers to be highly efficient in the collection and quantitation of bacterial cells and spores in aerosols of low to moderate concentrations (Bourdillion, Lidwell, and Schuster, 1948; DuBuy, Hollaender, and Lackey, 1945; Decker and Wilson, 1954; Kuehne and Decker, 1957; and Decker *et al.*, 1958). Sampling times and air volumes are both increased markedly over the maximum obtainable with liquid impingers.

If a suitable solid medium of sufficiently low melting point to permit liquefaction and dilution at temper-

atures tolerated by the virus could be used, the slit sampler would possess the theoretical capability of sampling viral aerosols.

It is the purpose of this paper to report on (a) the ability of the slit sampler to recover aerosolized virus particles when using gelatin collecting media, (b) the comparison of the slit sampler with the all-glass impinger for collection of aerosolized virus particles, (c) the effect of culture plate aeration on sampling virus aerosols with the slit sampler, and (d) the use of the slit sampler for simultaneous collection of bacterial and viral aerosols by modifying a standard culture plate and using two different collecting media.

MATERIALS AND METHODS

The slit sampler (figure 1) developed by Decker and Wilson (1954) was used in these studies to sample T-3 bacteriophage aerosols for 1-hr periods. This sampler is operated by drawing air by vacuum through the slit opening in the top of the sampler at a rate of 1 cu ft per min. The virus particles in the air are impacted on the surface of a solid gelatin medium contained in a plastic culture plate (150 by 20 mm), which is rotated at a uniform rate beneath the slit by a timer mechanism located in the base of the sampler. A 1-hr timer, which allowed the plate to make 1 revolution per hr, was used in this sampler.

Aerosolization of T-3 bacteriophage. *Escherichia coli* bacteriophage T-3 suspensions were produced in Petri dishes on a solid F medium (Adams, 1959). The lysates were harvested from the surfaces of the agar plates by washing with a few milliliters of distilled water. The lysates were purified by centrifugation at moderate

speeds to remove bacterial debris and larger particulate matter and finally by filtration through membrane filters.

The T-3 bacteriophage aerosol studies were conducted in a 1500-L Plexiglas chamber. Dynamic aerosols were produced by atomizing T-3 bacteriophage (2×10^{10} particles per ml) suspended in sterile, filtered water with a Vaponefrin nebulizer.¹ A temperature of 20 to 24 C and a relative humidity of 70 to 74 per cent were maintained inside the chamber. Particle size studies of the aerosols generated under these conditions showed that 96 per cent of the particles were in the size range of 0.5 to 1 μ .

Sampler evaluation. Slit samplers operating for 60 min and containing either a 6 per cent or a 12 per cent gelatin medium were used for collection of the test aerosol. For comparative purposes, four all-glass impingers (AGI-30) (Wolf *et al.*, 1959) with the stem orifice located 30 mm from the bottom of the impinger were used. These were operated consecutively for 15 min each over the same sampling interval as the slit sampler at 12.8 L per min.

The impinger collecting liquid was 20 ml of heart infusion broth plus 1 drop of Dow Corning² antifoam B to minimize foaming. The slit sampler collecting media were 6 and 12 per cent gelatin in 100 ml of phosphate buffer solution. The gelatin media were allowed to liquefy at 37 C for 30 min before assaying. The contents of the four AGI-30 samplers were pooled and a single assay of the aerosol concentration was obtained. All collecting media were assayed for T-3 bacteriophage by making serial dilutions in nutrient broth and plating 1-ml aliquots in triplicate, using the agar layer method described by Adams (1959). Fresh nutrient broth cultures of *E. coli* strain B, aerated on a shaking machine for 4 to 6 hr, were used for phage assays. The phage plaques were counted after incubation at 35 C for 7 hr, and the number of phage particles recovered per liter of air sampled was calculated for each sampler.

¹ The Vaponefrin Company, Upper Darby, Pennsylvania.

² Dow Corning Corporation, Midland, Michigan.



Figure 1. Slit sampler and culture plates

Effect of plate aeration on sampling T-3 bacteriophage. Three slit samplers, each containing solid 12 per cent gelatin medium, collected aerosol samples under the following conditions: (a) sampled for 15 min and aerated for 45 min, (b) aerated for 45 min and sampled for 15 min, and (c) sampled for 15 min with no aeration before or after sampling. The aeration was accomplished by drawing filtered air from the aerosol chamber through the sampler. Since three slit samplers were used in this experiment, all three procedures could be tested at the same time in the same aerosol, with the actual sampling of each procedure occurring simultaneously. The gelatin medium was assayed for T-3 bacteriophage in the same manner as described earlier.

Simultaneous collection of T-3 bacteriophage and *Bacillus subtilis* var. *niger*. The standard (150 by 20 mm) culture plate that is used in the slit sampler was divided into two sections by inserting a plastic annular ring in the center of the plate (figure 1). This modification enabled two different collecting media, one for viruses and the other for bacteria, to be used in the same plate. In this experiment, 12 per cent gelatin in phosphate buffer solution was used in the outer ring for the collection of T-3 bacteriophage, and nutrient agar was used in the inner ring for the collection of *B. subtilis* var. *niger*.

Three slit samplers sampled a mixed aerosol of T-3 bacteriophage and *B. subtilis* var. *niger* for 60 min at a rate of 1 cubic foot per minute. After sampling, the gelatin medium was removed from the plate and assayed for T-3 bacteriophage. The agar portion of the plate was incubated at 37 C for 24 hr and counted.

RESULTS

Comparison of the all-glass impinger and the slit sampler. Using the AGI-30 as unity, the results of 10 trials, summarized in table 1, show that the average per cent recovery for each slit sampler was 68.4 when 6 per cent gelatin was used and 75.3 when 12 per cent gelatin was used. In 9 of the 10 trials, the 6 per cent gelatin started to liquefy after 45 to 50 min of sampling. In all trials, the 12 per cent gelatin remained solid during the 60-min sampling period.

TABLE 1
Recovery of T-3 bacteriophage aerosols with slit and AGI-30 samplers

Sampler	Media	Avg Per Cent Recovery Compared with AGI-30*
Slit, 1 hr.....	Gelatin, 6%	68.4
Slit, 1 hr.....	Gelatin, 12%	75.3
AGI-30†.....	Heart infusion broth	100

* Each figure represents an average of 10 trials.

† Four 15-min samples were pooled.

Effect of plate aeration on sampling T-3 bacteriophage.

The results of 15 trials indicate that 45 min of aeration either prior to or after the collection of a 15-min aerosol sample has no detrimental effect on the viability of the sampled virus. The recovery of virus during the last 15 min of sampling approximated the recovery during the first 15 min. These data are indicative of a consistent sampling efficiency over the 60-min sampling period.

Simultaneous collection of T-3 bacteriophage and B. subtilis var. niger. The results of 10 trials show that the slit sampler equipped with a divided plate to accommodate both a gelatin and an agar medium can be used to recover viral and bacterial particles simultaneously from a mixed aerosol over a 60-min sampling period.

DISCUSSION

These studies show that the slit sampler can be used to collect air-borne T-3 bacteriophage particles continuously for 1 hr on 12 per cent gelatin with a good collection efficiency. Recoveries of T-3 bacteriophage with the slit sampler are comparable to those of the high-velocity liquid impinger. The slit sampler collection efficiency is consistent over a 1-hr sampling period and once the virus particles are collected on the gelatin surface, continued sampling over the remainder of the plate has no effect on the virus already collected. When the slit sampler is equipped with a divided plate containing a gelatin and an agar medium, it is capable of collecting, with good efficiency, a mixed viral and bacterial aerosol.

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SUMMARY

Slit samplers have been used to collect an aerosol of T-3 bacteriophage continuously for 1 hr on 12 per cent gelatin with good efficiency. The collection efficiency is consistent over a 1-hr period. A 45-min aeration period has no detrimental effect on the virus already collected, and no dehydration of the 12 per cent gelatin medium was observed. The slit sampler with appropriate media has been shown to possess a potential capability of sampling simultaneously a mixed viral and bacterial aerosol.

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ERRATUM

In the Microbiological Process Discussion, "An Approach to the Kinetics of Microbiological Deterioration," A. David Baskin and Arthur M. Kaplan, Appl. Microbiol., **8**, 1960, errors appear in two formulas on page 320. The correct formulas are:

$$E_{(v,t)} = \frac{E(x)}{\alpha - \beta} [\alpha e^{-\beta t} - \beta e^{-\alpha t}]$$

and

$$-\int (t_2 - t_1)$$