Method for Studying Particle Size and Infective Potential of Infectious Bovine Rhinotracheitis Virus Aerosols

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A technique is described for estimating the number of potential respiratory infectious loci of aerosolized infectious bovine rhinotracheitis virus.

The Anderson (1) aerodynamic particle sizing sampler was used in the past decade to collect and size bacterial particulates. Airborne particles are impacted directly on nutrient agar surfaces in six stages of aerodynamic sizes. Because of localized drying of the cell monolayer at the impaction site, it is implausible to collect virus particles directly on a cell monolayer. The usefulness of the Andersen sampler was extended to virus collection by Jensen (3), who washed the agar surface and assayed the washings for plaque-forming units (PFU). Guerin and Mitchell (2) and Wolfe et al. (4) proposed a gelatin collection surface which was melted and assayed. These procedures provide an estimate of PFU for each size category of the sampler but do not provide information as to the number of particulates involved. A single particle may contain many PFU but produce only one locus of infection. Alternatively, many particles may each contain few PFU, and thus a large number of loci could be produced from a small quantity of virus. These differences result not from variations in the virus but from dissimilarities in the manner in which it was aerosolized. A knowledge of these parameters is desirable in the careful study of dose response relationships of airborne infection.

A technique capable of providing an estimate of the number of infectious particles and hence an estimate of the number of potentially infected loci in the lungs is presented here. The technique consists of collecting the aerosol by jet impaction on agar-solidified tissue culture media. After collection the agar is inverted on a monolayer of susceptible cells thus forming an overlay.

Two strains in infectious bovine rhinotracheitis (IBR) virus were utilized in developing this sampling technique. Strain "V" is a vaccine strain originally obtained from Armour-Baldwin Laboratories. This particular strain was useful because it was a readily available attenuated virus and produced consistent plaque characteristics in testing the technique. The other strain, LY 985-3, was isolated from a field case of IBR at Utah State University.

Specific viral suspensions were aerosolized into a test chamber of 2 ft³ with a deVilbis clinical atomizer and equilibrated for 1 min. The virus laden particulates were collected from the test chamber by using an aerodynamic size model 30 (AIR, Inc., Logan, Utah). Molded glass collection plates filled with 27 ml of tissue culture media solidified with 1.5% Noble Agar (Difco) were used. The agar surface may be conditioned by storage for several days or by inverting the opened dishes on a sterile rack for a few hours before use in the sampler. This conditioning removes the surface water which tends to distort the impaction pattern. Water removal also assists in applying the agar surface to the monolayer without slippage.

Transfer of the agar from the collection plates to the cell monolayer is facilitated by cutting a ring in the agar ½ inch from the collection plate wall. A no. 11 surgical blade fitted with a gauge may be used for this purpose. The peripheral ring of agar is pulled from the corner of the dish while inverted. The agar disc is then held vertical and pried away from the glass plate with a sterile spatula. About one-third of the disc can be separated from the glass without danger of the agar falling.

The spatula is then carefully inserted under the agar as the plate is turned back to horizontal. The agar layer may then be transferred to the cell monolayer in a petri dish from which the liquid media have been aspirated. Any air trapped under the agar must be removed and can be done by stroking the agar with the spatula or a sterile swab.

Plaques appear as necrotic areas in the cell
monolayer when viewed with the low power microscope. The use of a petri dish clamp on a mechanical stage facilitates scanning the areas to be counted.

The results of aerosols generated from two virus suspensions are shown in Table 1. The distribution of counts among the six stages reflects the particle size distribution of the aerosol. The LY 985-3 suspension had a virus titer approximately 10 times that of strain "V."

Precautions against including areas of damaged cells in the plaque count should be observed. The immediate areas of the agar edge and bubble areas can be removed from consideration in the plaque count by use of counting masks made by punching holes of known size in self-adhesive paper stock. The masks can be placed on the petri dish over randomly selected areas that are not otherwise affected by the factors mentioned. For example, since the impaction areas of each stage of the sampler are approximately 7.07 square inches, a circular mask 1 inch in diameter represents 11.1% of the collection surface. Therefore, the average count of 1-inch masks should be multiplied by 9.0 to obtain an estimate of the number of positive impact areas per stage containing at least one PFU.

Characteristics of the model 30 aerodynamic sizer permit more than one particle to be collected on a specific impact area. This may result in a cluster of microplaques in one impact area. Such areas merge into single plaques with time and should be counted as one plaque. This estimate may then be transformed to a particle count estimate by use of a conversion table supplied with the instrument. This technique, therefore, estimates in each of six size categories the number of potentially infectious particles in the respirable air of experimental animals.

**LITERATURE CITED**

1. Andersen, A. A. 1958. New sampler for the collection, sizing, and enumeration of viable airborne particles. J. Bacteriol. 76:471–484.
2. Guerin, L. F., and C. A. Mitchell. 1964. A method for determining and concentration of airborne virus and sizing droplet nuclei containing the agent. Can. J. Comp. Med. Vet. Sci. 28:283–287.
3. Jensen, M. M. 1964. Inactivation of airborne viruses by ultraviolet irradiation. Appl. Microbiol. 12:418–420.
4. Wolfe, L. G., R. A. Griesemer, and R. L. Farrell. 1968. Experimental aerosol transmission of Yaba virus in monkeys. J. Nat. Cancer Inst. 41:1175–1195.

**TABLE 1. Example of stage distribution of two aerosolized virus suspensions**

| AIR, Inc. model 30 stage no. | Counts of virus particles |  |
|-----------------------------|---------------------------|---|
|                            | LY 985-3                  | Strain "V" |
|                             | Plaque count | Particle count (estimated) | Plaque count | Particle count (estimated) |
| 1                           | 88           | 99                       | 0            | 0                         |
| 2                           | 339          | 752                      | 20           | 21                        |
| 3                           | 400          | >2,427                   | 76           | 84                        |
| 4                           | 398          | 2,127                    | 158          | 201                       |
| 5                           | 386          | 1,341                    | 91           | 103                       |
| 6                           | 246          | 384                      | 22           | 23                        |