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Project Summary

Development and Evaluation of an Ambient Viable Microbial Air Sampler

Kerby F. Fannin and Stanley C. Vana

The purpose of this project was to enhance existing capabilities for quantitative detection of viable microorganisms in the air. The specific objectives were: (1) to review available methodologies for ambient viable microbial air sampling; (2) to design an ambient viable microbial air sampler; (3) based on a new or an existing design, to fabricate an air sampler; and (4) to evaluate the suitability of this sampler for detecting aerosols containing bacteria and viruses. In addition this study was intended to assess the applicability of various sampling methods to studies of specific environmental microbial aerosol problems.

To fulfill a need for a standard microbial large volume aerosol sampler, an air sampler design based on the principle of staged impaction was proposed. A major limitation of this sampler was that the microbial aerosol collecting substrate, consisting of a continuously wetted surface, required a considerable amount of development and optimization. Thus, to fulfill near-term needs for ambient viable microbial air sampling, the existing sampling concept of cyclone scrubbing was selected.

Studies were performed to evaluate the suitability of cyclone scrubber samplers and a continuously wetted substrate for detection of several types of microorganisms. The samplers were evaluated in a dynamic aerosol chamber using all-glass impingers as reference samplers. Comparison of a stainless steel and a glass cyclone scrubber sampler for detecting Bacillus subtilis var. niger spore aerosols of about 1.1 - 3.3 μm count median diameter showed no significant differences in their relative collection efficiencies. Consequently, based upon considerations such as ease of construction, present usage, and potential availability, the glass cyclone scrubber was selected for further evaluation. This sampler showed geometric mean relative collection efficiencies for B. subtilis var. niger spore aerosols of 52% and 68%, depending upon the composition of the disseminating fluid. In studies using different organisms, in similar sized aerosols, this relative efficiency was 46% for Serratia marcescens, 76% for f_2 coliphage, and 92% for poliovirus type 1. During the process of aerosolization and collection, the greatest viability losses in both the reference and cyclone scrubber samplers were observed with poliovirus, followed by f_2 coliphage, and S. marcescens.

Based on the studies, it was recommended that the methods used for the detection of low concentrations of ambient viable microbial aerosols be standardized, that a selected standard sampler be evaluated under a wide range of conditions for optimization of critical parameters, and that relative microbial aerosol evaluations be performed with a reference sampler having a sensitivity similar to that of the test sampler.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Cincinnati, Ohio, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

Project Objective

The overall purpose of this project was to enhance existing capabilities for quantitative detection of viable microorganisms in the ambient air. The specific objectives were: (1) to review available methods for ambient viable microbial air sampling; (2) to design an ambient viable microbial air sampler, (3) to fabricate an air sampler, based on a new or an existing design; and (4) to evaluate the suitability of this sampler for detecting aerosols containing bacteria and viruses. In addition, this study was intended to assess the applicability of various sampling methods to specific environmental microbial aerosol problems.

Rationale for Study

The extent of environmental exposure of human populations to infectious microbiological aerosols and the effects of such exposure on incidence of infectious disease have not been conclusively determined. Such determinations can be made, provided that reliable and sensitive methods are available to detect such aerosols or indices of exposure. Detection of indices of human exposure requires that members of a population serve as sentinels until an observable effect is demonstrated. Such human exposure can, however, be mitigated if contamination by infectious aerosols is detected early and reduced. The detection of infectious aerosols with both precision and accuracy is dependent upon the availability of adequate sampling and assay methodologies.

Numerous sampling techniques exist for detecting microbial aerosols. These techniques operate on a wide variety of principles and their use for applications requiring high sensitivity has not been standardized. Research in experimental infectious aerobiology has involved aerosol generation in static or dynamic chambers at concentrations detectable with conventional instrumentation, usually at relatively low air sampling rates and short operational time limits. Determination of low concentrations of microbial aerosols in the ambient outdoor environment, however, requires more sensitive instrumentation. Evaluation of the risk of exposure and potential infection with source-related infectious microbial aerosols often requires an extensive sampling program. The methods used for such sampling must be sufficiently sensitive to detect such aerosols at very low concentrations. This may necessitate sampling at relatively high air flow rates for long time periods. The applicability of available air samplers to ambient environmental aerobiological studies is determined by the inherently designed capabilities. Ideally, such a sampler should have a high collection efficiency, maintain the viability of collected microorganisms without permitting growth, discriminate between respirable and non-respirable particles and collect the sample so that it can be easily assayed. In addition it should be easily sterilized, highly reliable, simple to operate, and capable of remote-control operation, and its cost should be such that it can be used in routine monitoring programs.

Before undertaking the task of developing a new sampler, the available air samplers were examined to determin and select operational characteristics that would be applicable to the final design. Main emphasis was placed on air sampling devices used for detection of viable microbial aerosols. The principal aerosol collection concepts reviewed for their application to viable microbial aerosol sampling included sedimentation, filtration, impingement, precipitation, centrifugal separation, and impaction.

Experimental Approach

The research effort was performed as several consecutive task. The direction of each task was dependent upon the findings of previous tasks and program decisions made in consultation with the U.S. EPA Project Officer. The project tasks can be summarized as follows:

Review of Literature

A review of literature related to air samplers having application to the project objective was performed. The purpose was to determine the availability and suitability of existing instrumentation as samplers of ambient viable microbial aerosols.

Air Sampler Design

The need for a new air sampler design was based on the review of existing concepts of microbial air samplers. [illegible] design of the new air sampler was based on such criteria as estimated collection efficiency, sensitivity, reliability, ease of sterilization, viability of collected organisms, ease of sample assay, capacity for remote operation, particle size discrimination, and cost of construction and operation. The conceptual design was reviewed by several staff members of organizations designated by the U.S. EPA Project Officer.

Air Sampler Construction

Samplers based on existing designs were fabricated. The number of samplers constructed was sufficient to perform evaluation studies, and to provide additional samplers as required by the U.S. EPA Project Officer.

Air Sampler Evaluation

The sampler selected for further study was evaluated by comparing its performance in collection of viable and non-viable aerosols to that of a reference sampler. The purpose of these comparative studies was to determine the relative collection efficiency of the two samplers for the recovery of aerosols containing uranine dye, bacteria (*Bacillus subtilis* var. *niger* spores an[illegible] *Serratia marcescens*), and viruses ([illegible] coliphage and poliovirus type 1). This relative aerosol collection efficiency was expressed as:

$$CE = \frac{S}{(R_1 \times R_2)/2} \times 100 \quad (1$$

where

CE = relative aerosol collection efficiency

S = aerosol concentration as determined by test sample

R_1 and R_2 = aerosol concentration as determined by paired reference samplers at pre-S[illegible] position.

The aerosol slippage through the test sampler was determined by comparing the chamber aerosol concentrations detected with reference samplers located at the pre- and post-sample positions. The pre-sampler position was upstream from the test sampler while the post-sampler position was downstream. The percent survival of bacteria and viruses during the aerosolization and sampling process was determined relative to *B. subtilis* var. *niger* spore aerosolized simultaneously with th

test organism. The percent survival was calculated as follows:

$$PS = \frac{T_1}{B_1} \times \frac{B_o}{T_o} \times 100 \qquad (2)$$

where

PS = percent survival
B = *B. subtilis* var. *niger* concentration in the spray suspension (B_o) or in the aerosol sampler (B_1)
T = test organism concentration in the spray suspension (T_o) or in the aerosol sampler T_1).

The method of sampler operation, including the sampling medium used, air sampling rates, duration of collection, and sampling fluid flow rates, was typical of that used in field studies and did not necessarily include an evaluation of multiple methods of operation. The comparative studies were performed in a dynamic aerosol chamber system using aerosol concentrations of dye or microorganisms that were detectable with both the test and reference samplers. The aerosol particle size, >1 μm count median diameter (CMD), was similar to that which might be observed in field studies of source-related ambient viable microbial aerosols.

Continuously Wetted Substrate Evaluation

A continuously wetted substrate was evaluated in a test stand device to determine its potential suitability for use in a viable microbial aerosol impacting-type sampler. The substrate was evaluated to determine:

The recovery of *B. subtilis* var. *niger* spores after collection in nutrient broth and transferring the membrane surface to nonselective nutrient agar;

Its applicability for recovery of total coliform (*Enterobacter aerogenes*), fecal coliform (*Escherichia coli*), and fecal streptococcus (*Streptococcus fecalis*) by plating onto selective media; and

The performance of the substrate-containing device compared to that of a slit sampler for recovery of *B. subtilis* and *S. marcescens*.

Conclusions

1. There is a need for standardization of methods for detecting low-level ambient viable microbial aerosols.
2. An air sampler based on the principle of staged impaction, with aerosol classification into respirable and non-respirable size ranges, may be superior to existing samplers.
3. A major limitation of this sampler design, however, is that the substrate for final microbial aerosol collection required further development and optimization prior to use in such a sampler.
4. A continuously wetted surface developed as a potential collecting substrate is suitable for collecting bacterial aerosols. Additional studies are needed to determine operating conditions optimal for survival of collected organisms on such a surface.
5. Until the collection substrate is optimized and the sampler design concept developed, an existing sampler, such as a cyclone scrubber, should be used for viable microbial aerosol studies where low concentrations are expected.
6. The existing sampling concept that demonstrates the greatest overall superiority, in terms of the criteria used for sampler evaluation, is that of cyclone scrubbing.
7. Comparison of a glass and a stainless steel cyclone scrubber showed that there was no significant difference in their relative efficiencies for collecting *B. subtilis* var. *niger* spore aerosols.
8. Further studies with the glass cyclone scrubber showed that the geometric mean relative collection efficiency for 1 to 3.5 μm CMD aerosols containing *B. subtilis* var. *niger* spores was 52% and 68%, depending upon the aerosol composition. The efficiency for *S. marcescens* was 46%, for f_2 coliphage 76%, and for poliovirus type 1 92%.
9. *S. marcescens,* f_2 coliphage, and poliovirus type 1 showed viability losses during the aerosolization-sampling process. The survival of poliovirus was less than or equal to 1% of that observed for the coliphage in samples collected with all-glass impinger reference samplers and with a glass cyclone scrubber.
10. Because of the great disparity between the air volumes sampled with the all-glass impinger and the cyclone scrubber test samplers, substantial fluctuations in relative aerosol collection efficiencies can be observed.

Recommendations

1. Methods used for detecting low-level ambient viable microbial aerosols should be standardized. These methods should employ a sampler that is specifically designed for this application, and that is reliable, robust, and can be effectively sterilized by conventional methods. Until such a sampler is developed, an existing device such as a cyclone scrubber sampler, should be used.
2. The standard sampler should be evaluated under varied and controlled conditions to optimize collection efficiency by selecting appropriate collecting media for the organisms being studied and by controlling critical parameters such as fluid flow and air sampling rates.
3. These evaluations should employ a viable microbial aerosol reference sampler that operates at an air sampling rate similar to that of the device under evaluation

Kerby F Fannin is with IIT Research Institute, Chicago, IL 60616, and Stanley C Vana is with the Institute of Gas Technology, Chicago, IL 60616
***Walter Jakubowski** is the EPA Project Officer (see below)*
The complete report, entitled "Development and Evaluation of an Ambient Viable Microbial Air Sampler," (Order No PB 82-113 689, Cost $11 00, subject to change) will be available only from
National Technical Information Service
5285 Port Royal Road
Springfield, VA 22161
Telephone 703-487-4650
The EPA Project Officer can be contacted at
Health Effects Research Laboratory
U S. Environmental Protection Agency
Cincinnati, OH 45268

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