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13. ABSTRACT

Describes a method for qualitatively and quantitatively estimating airborne microorganisms in a tropical environment. Identifies and describes facilities and equipment required. Provides procedures for calibration of airflow through membrane filter, air sampling, sample preparation, and microorganism counting and identification. Applicable to wet-hot and wet-warm climates.

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Gungus Test						
Tropic Environmental Test						

II

U. S. ARMY TEST AND EVALUATION COMMAND
COMMON ENGINEERING TEST OPERATIONS PROCEDURE

AMSTE-RP-702-107
Test Operations Procedure 8-2-514

28 March 1972

MICROBIOLOGICAL AIR SAMPLING IN THE TROPICS

SECTION I
GENERAL

1. Purpose and Scope. This procedure describes a method for collecting airborne microorganisms in wet-hot and wet-warm environments. These microorganisms can be counted and identified to estimate numbers and kinds present in the air at any area of interest. These data can be used for estimating the kinds and numbers of microorganisms to which test items are exposed and identifying the source of microbial contamination. This method is applicable in wet-hot and wet-warm climates, as specified in AR 70-38 (reference 1). It is not reliable in climates of lower absolute humidity.

2. Background. The impact of microorganisms on materiel testing in the tropic is undeniable. The presence of both great numbers and kinds of microorganisms in soil, air, water, and on vegetation surfaces makes it a virtual certainty that test items will be exposed to microbial activity during tropic environmental testing. Airborne fungal and bacterial spores and fungal mycelial fragments are responsible for the establishment of most of the microbial growth to which test items are subjected. A major method of sampling airborne microorganisms involves trapping individual cells on some surface, often a filter. The surface is then incubated in the presence of nutrients where the trapped cells grow to colony size. Thus, each colony which can be counted and identified represents a living microorganism taken from the air.

Many variations in this technique were tried by the U.S. Army Tropic Test Center in the study entitled "Environmental Data Base for Regional Studies in the Humid Tropics," (references 2 and 3). The important finding in these trials was that air drawn directly through a dry, sterile membrane filter gave the highest yields in numbers of microorganisms and the most consistent results. The method is simple, well suited for field use, and has been tested in wide scale applications in the Panama Canal Zone.

Sampling in temperate climates with this method is generally unreliable, however, giving low yields in numbers. This is probably due to the relatively dry air passing through the filter, desiccating the already trapped microorganisms and killing them.

Seasonal and diurnal fluctuations in airborne microorganisms are well documented and the number of airborne microorganisms varies greatly before and after a rain. Also, even with the best sampling technique not all microbes collected will remain viable and with the following method they would not be counted. The method is useful for collecting the microorganisms present in the air being sampled, but confounding factors such as the rainfall previously mentioned make interpretation of the quantitative data very difficult. Also, very few microorganisms grow and reproduce in the atmosphere. Their habitat and method of dispersal will determine their relative numbers and frequency of occurrence in the air so that although many microorganisms could contaminate test items from other sources than the air, there are many cases where airborne transmission is the predominant mechanism of contamination.

More direct methods such as microbiological field inspections and/or chamber testing can determine the vulnerability of test materiel to microbial attack with some certainty when followed by destructive testing techniques. An indirect ecological approach, such as the present method of taking a census of airborne microorganisms, can be very valuable, however. For example, an application of this method would be to sample air at a vehicle or aircraft test area to determine if microbial growth fouling fuel lines entered the system from the air, from reservoir tanks, or from some other source. The present method is also applicable to those tests in which design constraints do not permit visual inspection or swabbing of internal components.

3. <u>Equipment and Facilities.</u>

a. Biological laboratory equipped with:

(1) Autoclave

(2) Microscopes

(3) Balances

(4) Incubator

(5) Petri dishes

(6) Other incidental and expendable items

(7) Microbiological media

b. Air/vacuum pump

c. Standard flowmeter

d. Applicable chemicals for culture media

e. Membrane filters of 0.47mm pore size

f. Membrane filter holders

SECTION II
TEST PROCEDURES

4. Calibration of Airflow.

a. Objective. This procedure provides a method of metering airflow through the membrane filter. The exact volume of air passing through the filter must be known for any quantitative sampling.

b. Method. A critical orifice is used in this procedure to control airflow. It was chosen for its simplicity and lack of bulk in field usage. The critical orifice will keep airflow through the membrane filter at a constant, desired rate. This is based on the fact that the velocity of a gas flowing through an orifice will reach a maximum when the absolute upstream pressure with respect to the orifice is approximately twice the absolute downstream pressure. When a vacuum is applied to the downstream side of the orifice the flow through it will increase until the critical pressure ratio (1:2) is reached. A further increase in vacuum will not change the flow rate provided upstream conditions are unchanged, and as long as the downstream pressure is always less than half the upstream pressure. This theory applies to orifices in a flat, thin surface, but for practical purposes a capillary tube inserted in the vacuum line between the filter and the vacuum source accomplishes the same end. The capillary tubing should be 2 inches long with an outside diameter large enough to fit snugly in the vacuum tubing and an inside diameter of a size to give the desired flow rate. An orifice inside diameter of approximately 1.25 millimeters allows a convenient flow rate of approximately 11.5 liters per minute.

The exact flow through this orifice must be measured in the same arrangement that it will be used in the field. To calibrate the orifice, place it in the vacuum line between the vacuum pump and filter holder with the filter in place. Connect a standard flowmeter to the inlet face of the filter holder. To accomplish this, connect the broad end of a cone-shaped cover to the filter holder so that the entire filter surface is sealed within the cone. The narrow end is connected by rubber tubing to the flowmeter. Connect a mercury manometer across the orifice and adjust the vacuum source so the pressure differential is at least 380 millimeters (figure 1), and record the flow rate in liters per minute from the flowmeter. Once the orifice is calibrated the flowmeter and manometer are no longer needed. The flow rate through the filter will always be the same provided the pressure differential is always 380 millimeters mercury or greater.

c. Data Required. Record of the flow rate from the flowmeter in liters per minute for each orifice calibrated.

d. Analytical Plan. None

5. Air Sampling.

a. Objective. This procedure provides a method of collecting microorganisms from air.

b. Method. The filter holder, membrane filter, and a filter paper pad which supports the thin membrane filter must be sterile before sampling. The filter holder should be wrapped in kraft paper and autoclaved at 121°C. for 15 minutes. If the membrane filters and pads are not sterile, they are sterilized in the same manner. Using sterile technique, a pad and then a filter should be placed in each filter holder. It is important to emplace the filter after the holder is autoclaved because it often adheres to the holder and is then difficult to remove intact. For field sampling, if a portable electric generator is used for powering the vacuum pump, the sampling should be done at least 15 meters from the generator. The vacuum source, orifice, and filter holder with pad and filter in place are connected in line. The operator should stand downwind from the sampler. The filter holder is held or positioned so that the surface of the filter on which the microorganisms are trapped is 1 meter above and facing the ground (figure 2) within 5 to 10 meters of the test item. The kraft paper, or other means used to keep the filter sterile, should then be removed and the vacuum applied. This starts the sampling period. A 5-minute sampling period generally traps enough microorgamisms for a representative sampling, but not so many that crowding makes counting the colonies difficult. Increasing or decreasing sampling time will increase or decrease the numbers of microorganisms collected. Between 50 and 100 microorganisms can be collected on a 47-millimeter diameter membrane filter without crowding, thus avoiding confusion in counting and identification.

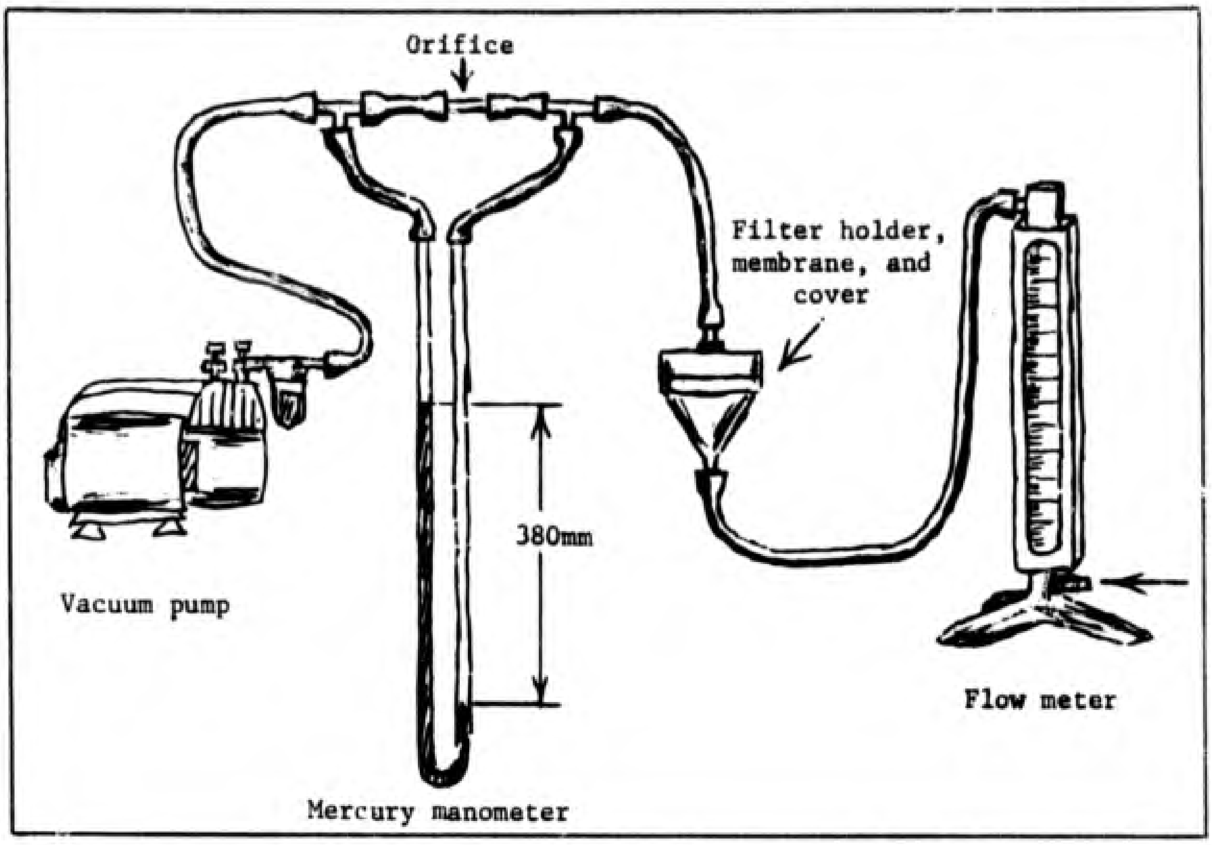

Figure 1. Calibration Configuration

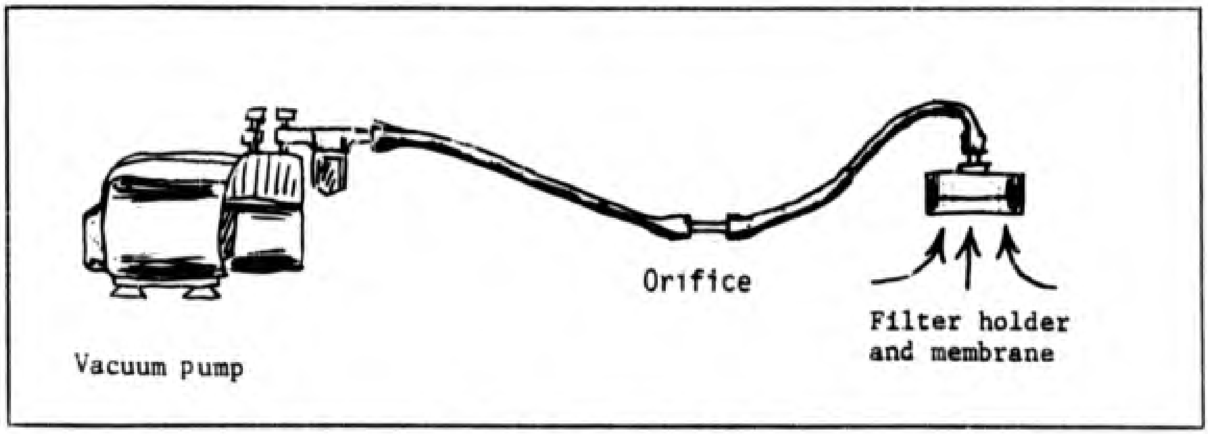

Figure 2. Sampling Configuration

c. Data Required.

(1) Location of sampling

(2) Length of sampling period in minutes

(3) Time of sampling

d. Analytical Plan. None

6. Sample Preparation.

a. Objective. This procedure outlines preparation of the collected microorganisms for counting and identification.

b. Method. After the sample is taken, using sterile technique, the filter is removed and placed on a sterile petri dish containing an appropriate solidified nutrient medium, with the surface holding the microorganisms facing away from the nutrient surface.

Excellent results with this method were obtained by using carrot infusion agar as the nutrient medium (see Section III, below). The carrot infusion agar gave good results supporting growth of fungi, actinomycetis, and many aerobic bacteria. For more complete isolation of bacteria, both aerobic and anaerobic nutrient agar were used successfully. The nutrient agar contained 3 grams of beef extract, 5 grams of peptone, and 15 grams of agar per liter. The group of microorganisms being sampled will determine the particular nutrient medium used.

The agar plate is incubated at 27°C for 96 hours (4 days) under aerobic conditions for the fungi, actinomycetes, and aerobic bacteria. For anaerobic bacteria, the incubation period and temperature are the same but anaerobic conditions are maintained during incubation.

c. Data Required. None

d. Analytical Plan. None

7. Counting and Identification.

a. Objective. This procedure determines the numbers and kinds of microorganisms collected.

b. Method. Using standard microbiological techniques, the individual colonies on the membrane filters are counted and identified. Bergey's Manual of Determinative Bacteriology (reference 5) is the basic tool used for the identification of unknown bacteria and actinomycetes. The unknown fungi are identified using Illustrated Genera of Imperfect Fungi (reference 6) as the primary guide.

c. Data Required.

(1) Number of colonies growing on the filter surface.

(2) List of the microorganisms identified and the numbers of each.

d. Analytical Plan. The following equation gives results in number of microbes/100 liters of air and represents an estimate of the airborne microorganism density at the area the sample was taken.

$$\frac{\text{microbes counted}}{\text{flow rate } \frac{(\text{liters})}{(\text{minute})} \times \text{sampling time (in min)}} \times 100 = \frac{\text{microbes}}{\text{100 liters of air}}$$

SECTION III
SUPPLEMENTARY INSTRUCTIONS

To prepare the carrot agar, place 30 grams of diced raw carrot in 250 milliliters of distilled water and autoclave at 121°C. for 15 minutes. Place 20 grams of agar in 750 milliliters of distilled water and autoclave at 121°C. for 15 minutes. Then, add the carrot juice to the agar solution and reautoclave.

Recommended changes to this publication should be forwarded to Commanding General, U.S. Army Test and Evaluation Command, ATTN: AMSTE-TP Aberdeen Proving Ground, Maryland 21005. Technical information related to this publication may be obtained from the preparing activity, Commanding Officer, U.S. Army Tropic Test Center, ATTN: STETC-PD-M, Fort Clayton, Canal Zone. Additional copies of this document are available from the Defense Documentation Center, Cameron Station, Alexandria, Virginia 22314. This document is identified by the accession number, AD __________, stamped on the first page.

APPENDIX
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