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BACTERIAL AEROSOL SAMPLER EVALUATION

WILLIAM F. HILL, CAPT., USAF (MSC)
CHARLES M. COX
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APRIL 1959

WRIGHT AIR DEVELOPMENT CENTER
AIR RESEARCH AND DEVELOPMENT COMMAND
UNITED STATES AIR FORCE
WRIGHT-PATTERSON AIR FORCE BASE, OHIO

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PROJECT NO. 7165
TASK NO. 71837

WRIGHT AIR DEVELOPMENT CENTER
AIR RESEARCH AND DEVELOPMENT COMMAND
UNITED STATES AIR FORCE
WRIGHT-PATTERSON AIR FORCE BASE, OHIO

McGregor & Werner Midwest Corp., Dayton, O.
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FOREWORD

This report was initiated by the Physiology Branch, Aero Medical Laboratory, Wright Air Development Center, Wright-Patterson Air Force Base, Ohio. The study upon which this report is based was accomplished by the Biological and Chemical Warfare (BW/CW) Defense Section under Project No. 1081, "BW/CW Vulnerability Assessment," Task No. 71815, "BW/CW Vulnerability Assessment, Aeromedical." The senior author, William F. Hill, Captain, USAF (MSC), of the BW/CW Defense Section* was project officer in charge of the over-all study. The field study was conducted from 14 May 1957 through 12 September 1957.

Included among those who cooperated in the study were Dr. A. R. Stanley, Chief, BW/CW Defense Section; Captain H. G. Vielhauer, and 1st Lt. Prell C. Vickers of the Aero Medical Laboratory, WADC.

* The BW/CW Defense Section has been deactivated. The BW/CW defense operation is now part of the Biochemistry Section.

ABSTRACT

The urgency for an interim bacterial aerosol detection device is reflected in the need for an adequate means of defense against biological warfare attack. ~~This study was set up as~~ a preliminary survey to evaluate several existing bacterial aerosol sampling devices in regard to their ability to consistently and reliably detect the presence of an artificially induced bacterial aerosol in the atmosphere. The candidate sampling devices evaluated in this study included several slit samplers, millipore filter samplers, all-glass impingers, and multi-stage Andersen Samplers. The results of this study indicated that, of the different sampling devices evaluated, the 6-stage Andersen Sampler and the 4-stage modified "Andersen" Sampler were superior in terms of consistency and reliability to detect the presence of the artificially induced bacterial aerosol in the atmosphere.

PUBLICATION REVIEW

This report has been reviewed and is approved.

FOR THE COMMANDER:

ANDRES I. KARSTENS
Colonel, USAF (MC)
Ass't. Chief, Aero Medical Laboratory
Directorate of Laboratories

INTRODUCTION

Many avenues of research and development are currently being pursued for the sole purpose of developing an efficient and reliable detector of induced bacterial aerosols. This work is being undertaken to provide a means of defense against biological warfare in which candidate agents will be disseminated by aerosolization. Other governmental agencies are concentrating their efforts on the basic research and development problem areas. Since aerosol detection is a function of sampling equipment, it becomes logical and worthwhile to set up a study to evaluate the several, existing, aerosol sampling devices already developed for bacteriological aerosol field assays. The selection of the various samplers used in this study was predicated upon their availability for study. It is expected that this study will continue as new and improved sampling devices are made available. In essence, this study is a beginning and should represent the initial concrete effort to provide a suitable, interim, bacterial aerosol detection device for the Air Force. The urgency for a device of this nature is reflected in the need for an adequate means of defense against biological warfare attack—the first line of defense resting in timely and efficient detection capability.

The evaluation of the candidate bacterial aerosol sampling devices used in this study was based upon the relative efficiency (consistency) and reliability of a particular sampler to detect the presence of a known, induced, bacterial aerosol cloud in the atmosphere.

MATERIALS

1. The candidate aerosol samplers used in this study and the modified Tokheim generator used for dissemination are illustrated in figures 1 through 9. The sampling devices consisted of multistage Andersen samplers, various types of slits samplers, and the Porton all-glass impinger.

2. The field setup utilizing sampler stands, aerosol generator, and vacuum pumps is diagrammatically presented in figure 10 and illustrated in figures 11 and 12.

3. Smoke pots (Stock No. 1325-219-8512) were used throughout the study for determination of wind direction and wind speed.

4. The test organism used was a 4-day culture of Bacillus globigii, (Bacillus subtilis var. niger), heat-shocked at 80° C. for 15 minutes. The test organism was prepared by inoculating 250-ml. quantities of Difco Tryptose Broth. Stock culture assays were conducted using spread-plate and pour-plate techniques.

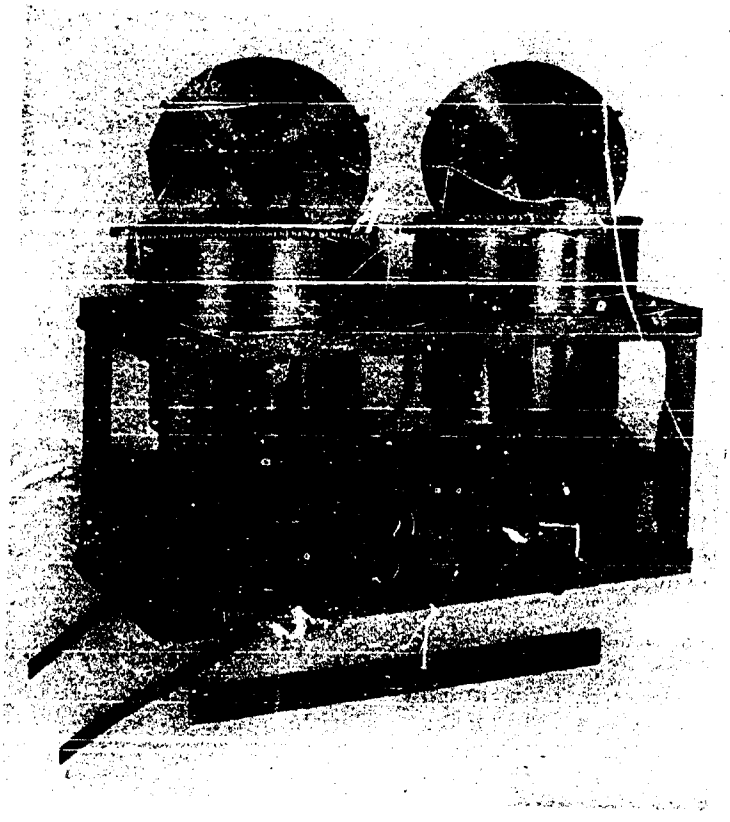


Figure 1 (left). Experimental airborne bacterial slit sampler. Designated D and D_1 in Phase II

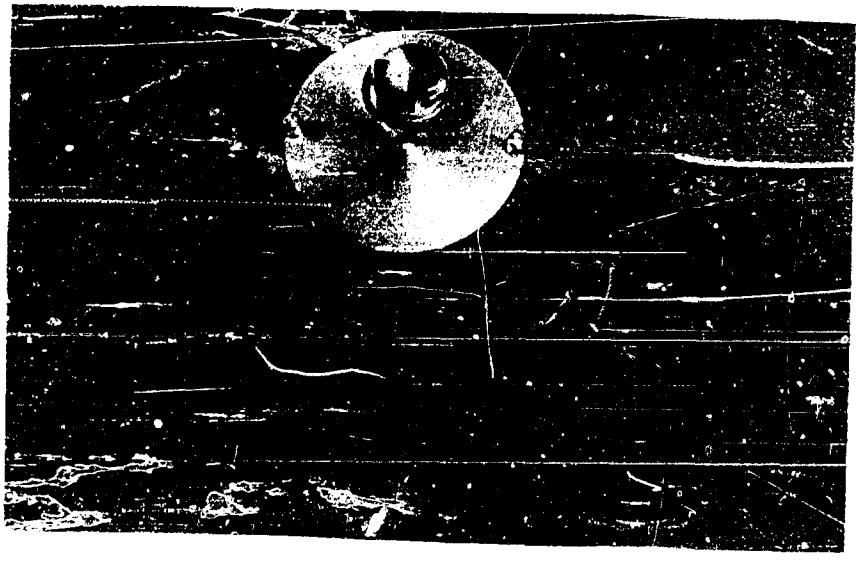


Figure 2 (above). Slit sampler. Designated B in Phase I and Phase II

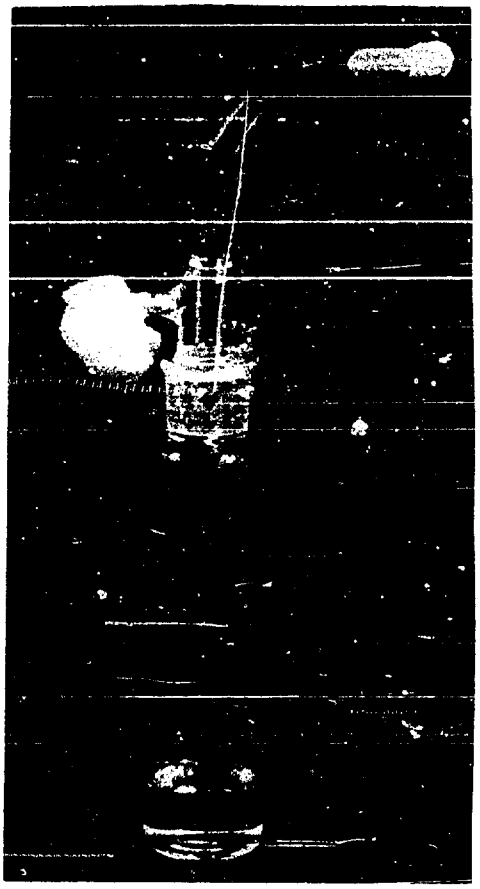


Figure 3 (above). Porton all-glass impinger.
Designated G and H in Phase I and Phase II

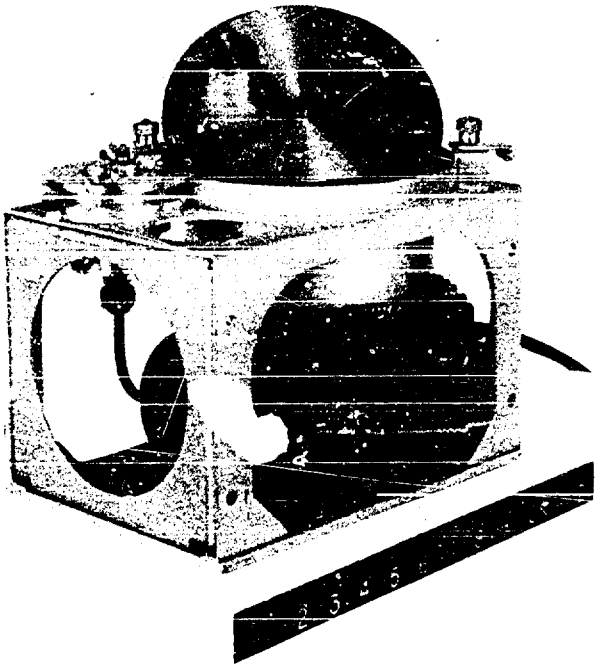


Figure 4 (above). Slit sampler and millipore filter sampler.
Designated C and small MF (C) in Phase II

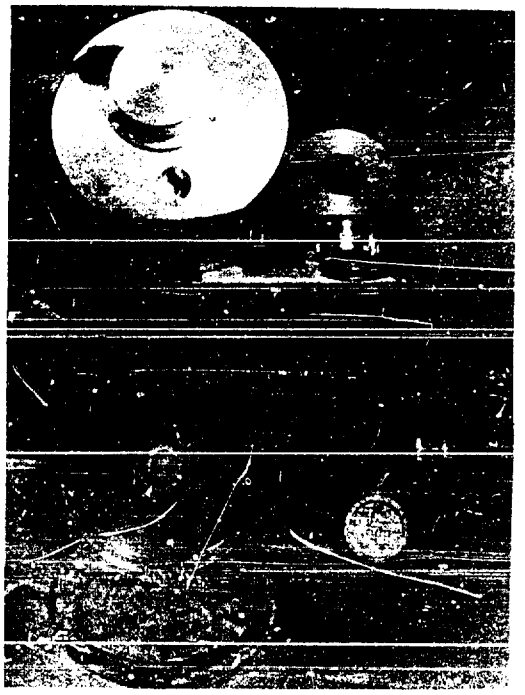


Figure 5 (right). Experimental 110 V. slit sampler and millipore filter sampler.
Designated A and small MF (A) in Phase I and Phase II

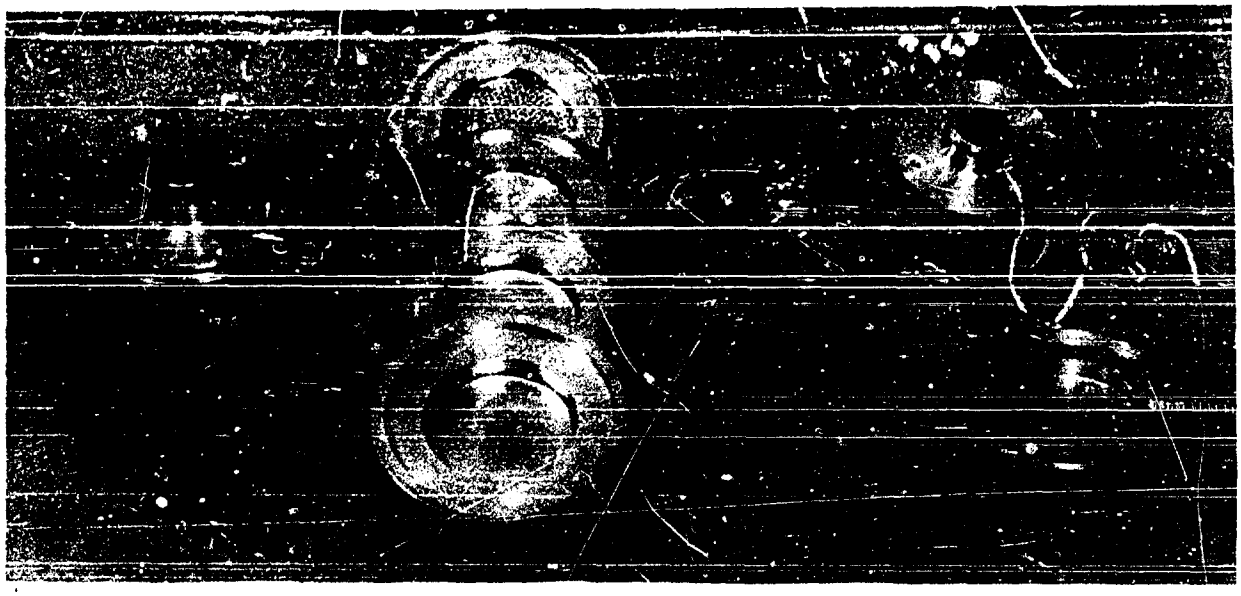


Figure 6 (above).
Four-stage modified "Andersen" sampler.
Designated E and F in Phase I and Phase II

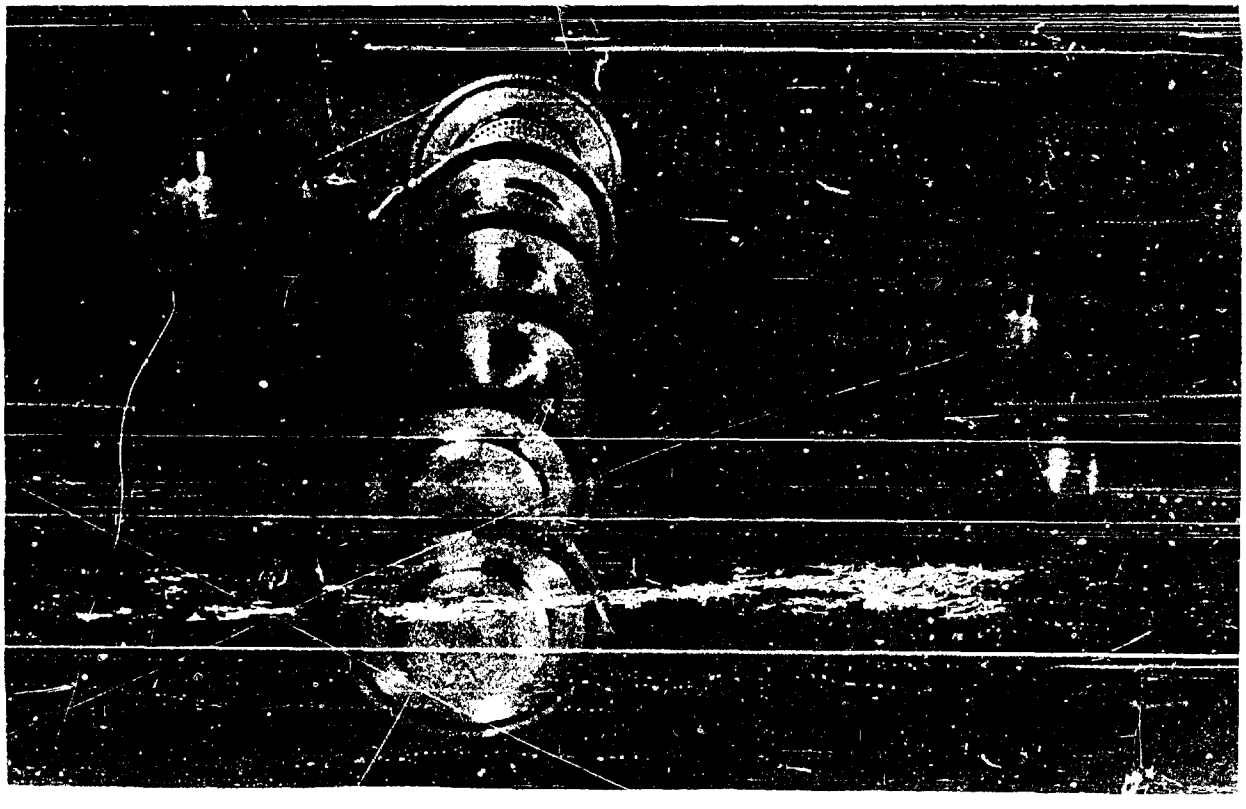
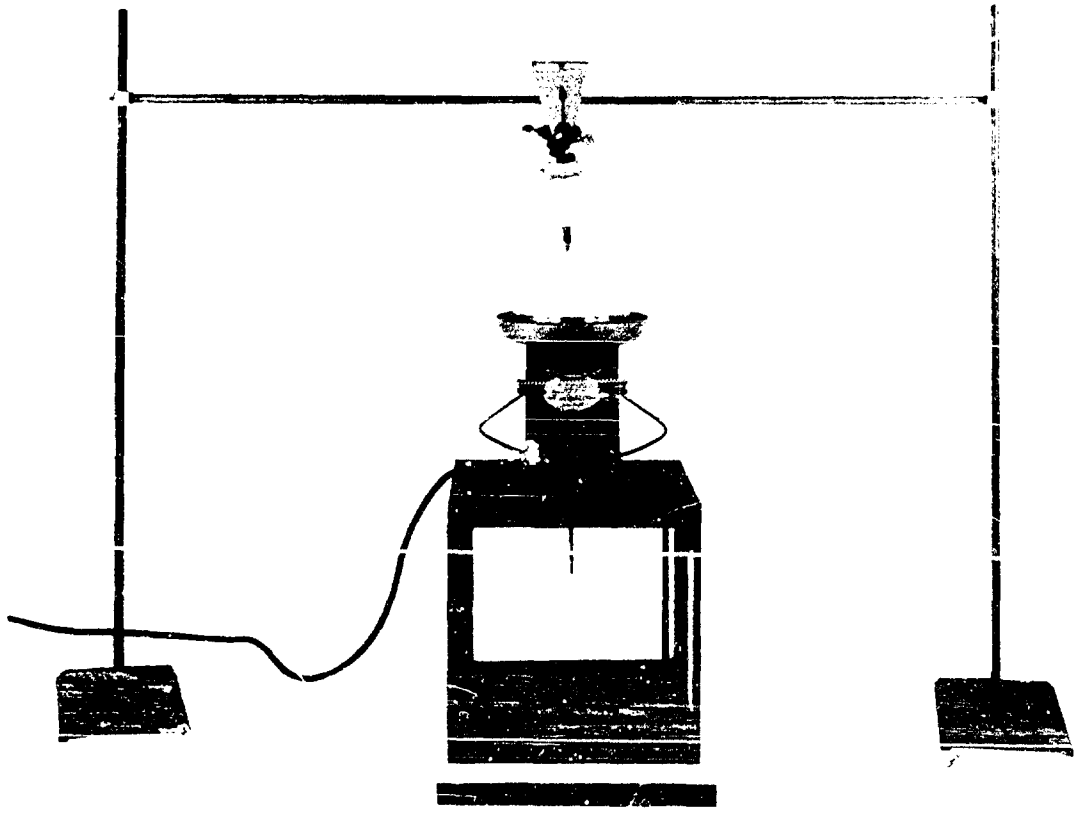


Figure 7 (above).
Six-stage Andersen sampler.
Designated C and D in Phase I

Figure 8 (left).
Modified Tokheim generator used in Phase I

Figure 9 (below).
Modified Tokheim generator used in Phase II



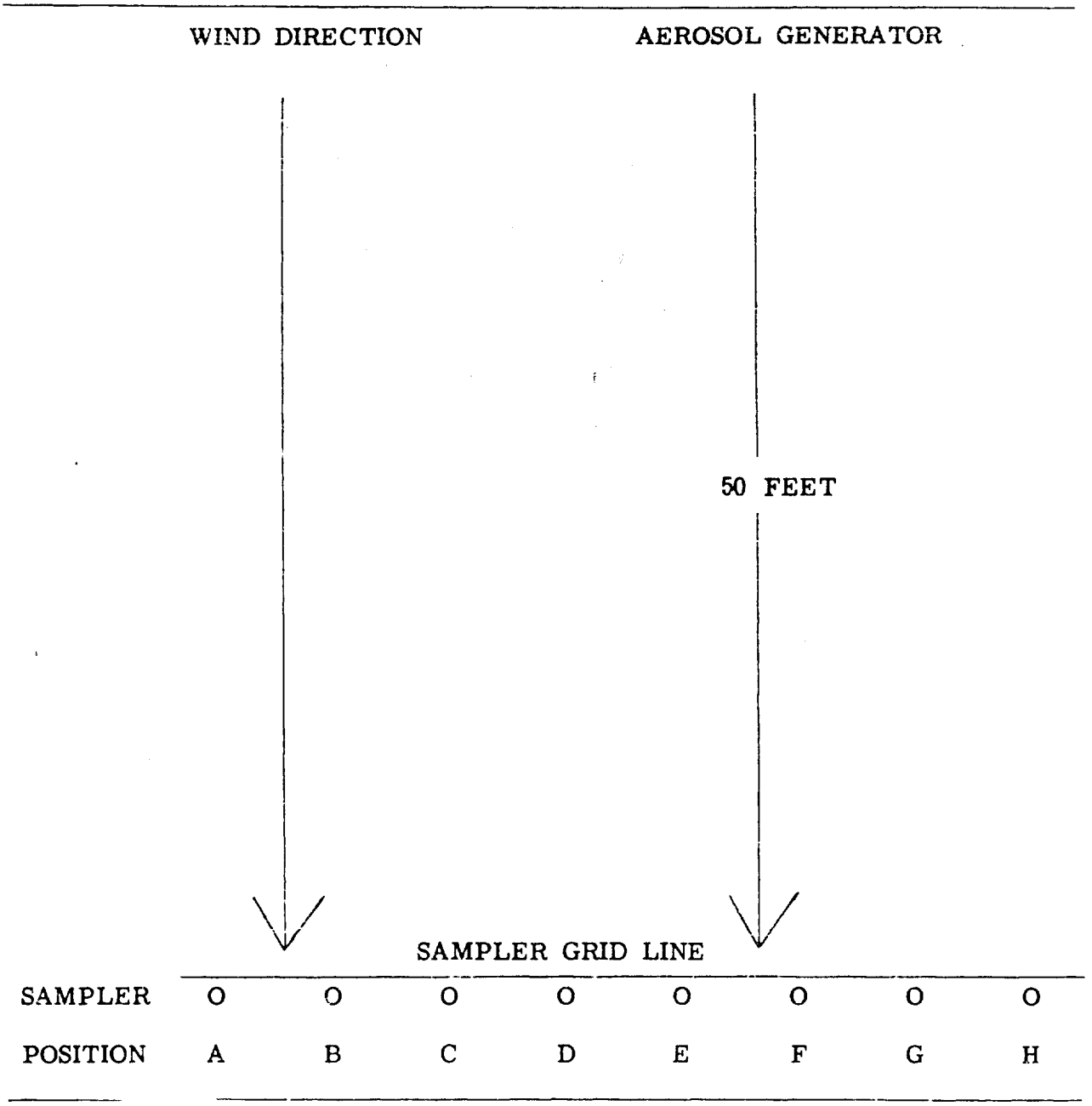


Figure 10. Diagrammatic Arrangement of Test Grid Setup

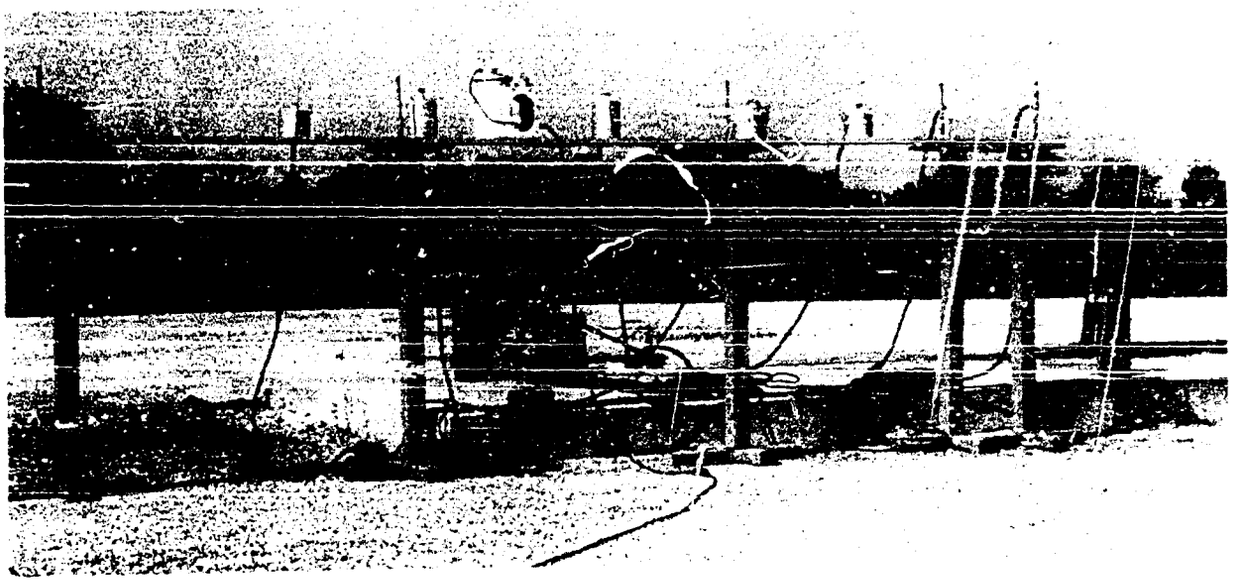


Figure 11 (above).
Sampler Setup

Figure 12 (above).
Test Grid Setup

METHODS

1. Phase I and Phase II each consisted of eight trials. The arrangement of samplers during each phase corresponded to the following 8 X 8 latin square in order to eliminate sampler position advantage:

SAMPLER ARRANGEMENT

Trial No.	
1	ABCDEFGH
2	BCAEFDHG
3	CADGHEFB
4	DFGCAHBE
5	EHBFGCAD
6	FDHAEGBC
7	GEFHCBDA
8	HGEBDACF

2. Prior to each trial, wind direction, wind speed, and relative humidity were taken. Temperature was recorded at ground level and at the sampler level (5 ft.) during each trial.

3. The sampling grid line was set up approximately 50 feet downwind from the aerosol generator (figure 10). Individual samplers were spaced on the sampler stands at least 1 foot apart.

4. Aerosol dissemination time in Phase I was 2 to 10 minutes with slurry output varying from 142 ml. to 222 ml. In Phase II, aerosol output was 150-ml. and 200-ml. volumes. In both phases, sampling time was 5 to 10 minutes, starting 30 seconds before dissemination of the culture suspension. The sampling time varied from one trial to another depending upon wind speed.

5. Results of each trial, consisting of colony counts, were recorded following a 24-hour incubation period of aerosol-collecting mediums.

RESULTS AND DISCUSSION

General

The results of this study are by no means definitive, for many questions still remain to be answered. However, certain valid impressions, based on the facts obtained, can be summarized and presented, thus providing information for agencies concerned with biological warfare defense problems insofar as detection of bacterial aerosols are concerned. As a point worth mention, it should be understood that the goal of this study was not one based on quantitativeness. For example, we were not primarily interested in the numerical tabulation of bacterial colonies, per se, for each sampling device. The only exception to this minor consideration was the evaluation of the reliability between duplicate sampling devices, in which statistical analyses were conducted on mean colony counts for the samplers by use of the "t" test. It was felt by our group that any extensive mathematical manipulations would not absolutely delineate detection capabilities of any particular sampling device. We were primarily interested in the relative efficiency of a particular sampling device to detect, qualitatively, the presence of a known, induced, bacterial aerosol in the atmosphere. This efficiency was measured by "detection consistency" which refers to the frequency of a sampler to detect the induced bacterial aerosol under a given set of circumstances inherent in daily meteorological variation. In addition, we were interested in the functional characteristics of a sampling device that had the ability to differentiate between the artificially induced bacterial aerosol and the bacteria, molds, and other microorganisms normally found in the air. This is important from the standpoint that the possibility exists that normal air microflora could "mask" the detection and identification of artificially induced bacterial aerosols.

PHASE I

The results of Phase I (see table I) indicate reliable consistency for some of the sampling devices and a lack of consistency in others. For example, the slit samplers and the Porton all-glass impingers, for the most part, failed to consistently detect the presence of the induced bacterial aerosol throughout the given set of circumstances for the eight trials. The reasons for the failure of the slit samplers to consistently detect the induced bacterial aerosol is not readily explicable. However, conjecture suggests that the operational characteristics of the slit samplers were probably responsible. It should be mentioned also that, in those trials in which the slit samplers did detect the aerosol, considerable "masking" of the specific bacterial colonies occurred as a result of normal-air, microflora overgrowth. The millipore filter samplers (small MF), for the most part, functioned very well and consistently picked up the induced aerosol. The only exception and lack of consistency with the small MF was in trials 6, 7, and 8 in which smoke particles

were collected (as a result of wind-direction change) and no bacterial colonies grew out—suggesting that smoke has an inhibitory effect.

The reasons for the failure of the Porton all-glass impingers to detect the induced bacterial aerosol are also not readily explicable. However, it is probably referable to the minimal number of bacteria in the disseminated aerosol and to the initial selection of the spread-plate technique for laboratory assay. In fact, in Phase II (see table II) this second "educated guess" was confirmed by changing the spread-plate technique to the pour-plate technique as the laboratory assay method.

The 6-stage Andersen samplers and the 4-stage modified "Andersen" samplers detected the induced bacterial aerosol on all the trials. In addition, mean value bacterial pickup for the eight trials of Phase I was the same for these two types of samplers, indicating similar consistency. Furthermore, statistical analysis of duplicate Andersen samplers confirmed the reliability of an individual sampler to detect the presence of the induced bacterial aerosol under all the conditions of this study with the same degree of consistency as its duplicate.

PHASE II

This phase was set up as a repeat of Phase I. However, certain modifications were introduced into the methods—viz., (a) a different method for disseminating the bacterial slurry was used (see figure 9 for view of modified Tokheim generator); (b) pour-plate laboratory assay of Porton all-glass impingers was used; and (c) slit samplers were paired, half receiving agar plates and half receiving large millipore filters. This latter comparison was set up to ascertain any relative differences in sampler impingement material.

In Phase II, for the most part, all samplers under test consistently detected the presence of the induced bacterial aerosol. One obvious exception, was the failure of the experimental 110-volt slit sampler. However, it was found that its sampling volume was less than the designated rate which could account for its poor detecting ability. In fact, this could also account for its failure to detect the aerosol in Phase I. Other slit samplers under test detected the induced bacterial aerosol consistently, but no similarity of mean counts was reflected between duplicate samplers, suggesting a lack of sampler reliability. In addition, as in Phase I, considerable masking of the induced aerosol occurred as a result of picking up normal-air microflora. However, we do not want to condemn the slit samplers, per se, because it is strongly felt in our group that the slit samplers used in this study did not readily lend themselves to the conditions under which this study was conducted. The Porton all-glass impingers reflected detection consistency throughout the eight trials. In addition, reliability was exhibited between the duplicate samplers. It is felt that the use of the pour-plate assay technique, in part, contributed to the successful operational evaluation of these samplers. However, it should

be pointed out the the all-glass impinger type of aerosol sampler would not serve as an interim aerosol detection device because of procedural difficulties inherent in its use. In fact, even its use as a bacterial aerosol-cloud assay tool seems to be questionable.

The millipore filter samplers (small MF) detected the bacterial aerosol consistently, but no comparison existed between mean colony counts——indicating a low level of reliability.

TABLE I

PHASE I

Samplers - Total Colony Counts per Sampler per Trial

Trial No.	A	B	C	D	E	F	G	H	Small MF (A)
1	7	0	19	32	28	33	0	1	4
2	11	0	27	75	48	42	0	0	26
3	0	0	22	13	30	42	0	0	3
4	0	0	6	9	1	3	0	0	2
5	2	0	186	191	81	174	0	1	102
6	0	0	92	104	90	80	1	0	0 (SMOKE)
7	5	24	78	80	58	97	0	0	0
8	0	5	9	12	6	8	0	0	0 (SMOKE)
Total Count	25	29	439	516	342	479	1	2	137
Mean	4.1	3.6	55	64.5	42.7	59.9	---	---	17.1

A= experimental 110 V. slit and small MF (A)
B= slit sampler (Agar medium)
C&D= 6-stage Andersen samplers
E&F= 4-stage modified "Andersen" samplers
G&H= Porton all-glass impingers

TABLE II

PHASE II

Samplers* - Total Colony Count per Sampler per Trial

Trial No.	A	Small MF (A)	B	C	Small MF (C)	D	D_1	E	F	G	H
1	0	2	12	6	16	0	10	2	12	10	7
2	0	4	8	4	17	5	7	10	16	6	6
3	0	16	14	7	36	3	5	25	20	11	6
4	0	20	0		90		16	232	188	27	36
5	1	66	12	73	151	37	35	32	63	13	8
6	0	16	14		149		3	62	1	8	3
7	3	6	9	24	26	4	6	1	9	1	7
8	4	7	8		1	6	11	19	19	6	3
Total Count	8	137	77	114	486	55	93	383	328	82	76
Mean Count	1	17.1	9.6	14.2	60.7	6.8	11.6	48	41	10.2	9.5

*A= experimental 110 V. slit sampler and small MF(A)
B= slit sampler (agar medium)
C= slit sampler (large MF sample)
D= slit sampler (agar medium)
D_1= slit sampler (large MF sample)
E&F= 4-stage modified Andersen samplers
G&H= Porton all-glass impinger

CONCLUSIONS

1. Under the conditions of this study, the 6-stage Andersen sampler and the 4-stage modified "Andersen sampler" exhibited "consistency" and reliability in detecting the presence of an artificially induced bacterial aerosol in the air, and as a result are considered to be superior to the other types of samplers subjected to the given set of circumstances comprising this study. In addition, other things being equal, these samplers could be used as interim detection devices for artificially induced bacterial aerosols.

2. The failure of slit samplers to detect consistently and reliably is not readily explicable, but mechanical and/or operational failure occurred during some of the testing trials. In addition, "masking" of the induced bacterial

aerosol by normal-air microflora is considered a major limitation of this sampling device for field work and precludes its consideration as an interim detection device for induced bacterial aerosols.

3. The Porton all-glass impinger consistently and reliably detected the induced bacterial aerosol in Phase II. However, time-consuming laboratory assay methods preclude its general use for field work. In addition, its use as an interim detection device is considered limited, essentially because of the unpredictability of predetermining bacterial concentrations in an aerosol cloud.

4. The millipore filter samplers (small MF) consistently detected the presence of the induced aerosol, but exhibited a lack of reliability between duplicate samplers. However, it should be acknowledged that collecting bacteria onto dry material is contraindicated since drying, as such, augments biological decay. Therefore, the millipore filter sampler is considered as a poor candidate for use as an interim detection device.

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