THE QUANTITATIVE ASSAY OF BACTERIAL AEROSOLS BY ELECTROSTATIC PRECIPITATION

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(With 8 Figures in the Text)

CONTENTS

INTRODUCTION

A great variety of instruments for the analysis of aerosol particles containing inanimate matter is available in which sedimentation, filtration, impingement, centrifugation and electrostatic, thermal or acoustic precipitation are employed.

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https://doi.org/10.1017/S002217240003744X Published online by Cambridge University Press

For the assay of bacterial aerosols, the methods generally used depend either on impingement, sedimentation and filtration or on centrifugation. The application of electrostatic, thermal and acoustic precipitation to bacterial aerosols, however, has only incidentally been investigated. Berry (1941) used a funnel device (Hollaender & DallaValle, 1939) combined with an electrostatic precipitator by the introduction of an ionizer consisting of fine wires on high voltage into the funnel. The sampling efficiency of the funnel device could be increased in this manner from 50 to 95%. Luckiesh, Taylor & Holladay (1946) described an electrostatic apparatus consisting of two parallel samplers of opposite charge which precipitate bacterial aerosol particles carrying an electric charge of their own. These two apparatuses did not find wide practical application. Valuable results were recently obtained with thermal precipitation by Orr, Gordon & Kordecki (1956); the volume of air analyzed was restricted to a maximum of 1·5 l. As far as we are informed, experiments on acoustic precipitation of bacterial aerosols have not yet been reported in the literature.

In this paper, the construction of two types of electrostatic precipitators is described. The results of sampling tests with bacterial aerosols carried out for the evaluation of the apparatuses in comparison with the slit sampler, membrane filters and impingers, are reported.

ELECTROSTATIC PRECIPITATION OF AEROSOLS

The electrostatic precipitation of aerosols is essentially a two step process in which charging of the particles is followed by precipitation in an electric field. The two steps of this process will be briefly discussed.

(1) *Charging of aerosol particles*

Most aerosol particles carry electric charges, depending mainly on the mode of generation. Their magnitude can be increased ten times or more in an unipolar corona discharge, by far the best means of particle charging.

Two distinct charging mechanisms, ion bombardment of the particles and thermal diffusion of ions to the particles, are present in the corona. Ion bombardment predominates in the charging of particles of more than 0·5μ diameter; smaller particles are mainly charged by thermal diffusion.

It has been shown (Pauthenier & Moreau-Hanot, 1932) that the saturation charge n_s obtained by spherical particles charged by ion bombardment can be expressed by

$$n_s = \frac{E_0 a^2}{e}\left(1+\frac{(D-1)}{D+2}\right) \text{ c.g.s. units of charge.} \qquad (1)$$

For conducting particles

$$n_s = \frac{3E_0 a^2}{e}. \qquad (2)$$

The saturation charge, which is determined by a balance between the repulsive field of the charge already accumulated on the particle and the driving force of the corona field, is proportional to both the field strength and the particle radius. This

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theoretical particle-charging equation has been confirmed by experiments of Fuchs, Petrjanoff & Rotzeig (1936) and White (1951). The charging time is inversely proportional to the concentration and mobility of the ions (White, 1951). The charging process is substantially complete in about 10^{-2} sec.

The saturation charge of particles smaller than $0{\cdot}5\mu$, which depends mainly on thermal diffusion of ions, is given by the approximate equation (White, 1951)

$$n_s = \frac{akT}{e^2} \ln\left(1 + \frac{aCN_0e^2}{kT}\, t\right) \text{ c.g.s. units of charge.} \tag{3}$$

In this process, ions are attracted by induction of image charges onto the particles. It will be noted that the saturation charge which depends on the absolute temperature, is independent of the field strength.

From equations (1) and (3) it follows that the charge of an aerosol particle increases with size and depends on field strength or temperature. Particles with a diameter of more than $0{\cdot}5\mu$ are charged predominantly by ion bombardment and smaller particles mainly by diffusion. Strictly speaking, however, at room temperature and at field strength from 1 to 10 kV./cm. only particles smaller than $0{\cdot}2\mu$ diameter are charged by ionic diffusion; charging of particles greater than $1{\cdot}0\mu$ diameter is exclusively due to ionic bombardment, but both diffusion and bombardment are operative in the charging process of particles with a diameter varying from 0·2 to $1{\cdot}0\mu$.

(2) *Precipitation of charged aerosol particles*

The precipitation of the charged particles is accomplished by the Coulomb force exerted by the electric field. The precipitation velocity (w) of a charged spherical particle is given by Stokes's law

$$n_s e E_0 = 6\pi\eta a w,$$

$$w = \frac{n_s e E_0}{6\pi\eta a}. \tag{4}$$

(3) *Collection efficiency in a cylindrical electric field*

In a cylindrical electric field, the gas flow is highly turbulent due to electrical wind effects of the corona. It has been shown (Deutsch, 1925) that these conditions lead to a statistical type of expression for the collection efficiency E

$$E = \frac{c_0 - c_L}{c_0} = 1 - e^{-2wL/Rv}. \tag{5}$$

Sometimes it is more convenient to use penetration (P) instead of E, the penetration being defined as

$$P = 1 - E = e^{-2wL/Rv}. \tag{6}$$

CONSTRUCTION AND DESIGN OF ELECTROSTATIC PRECIPITATORS

Two types of electrostatic precipitators were constructed for the analysis of bacterial aerosols. In one apparatus aerosol particles are precipitated on an agar layer, in the other a film of water forms the receiving electrode.

(1) *Construction of the precipitation apparatus*

The precipitator with agar collector consists of a glass tube coated with a nutrient agar-layer which enables a direct enumeration of colonies formed on incubation; the micro-organisms deposited on agar are protected against toxic effects of ozone (cf. Elford & van den Ende, 1942). The high-tension electrode is centred in the cylindrical collector, the agar layer being at earth potential. A positive corona is applied to the central electrode since it is known that positive ionization produces about ten times less ozone than negative ionization. A diagram of the collector tube is shown in Fig. 1. Fig. 2 demonstrates a collector tube after electrostatic precipitation of a *Serratia marcescens* aerosol and subsequent incubation at 25° C. for 30 hr.

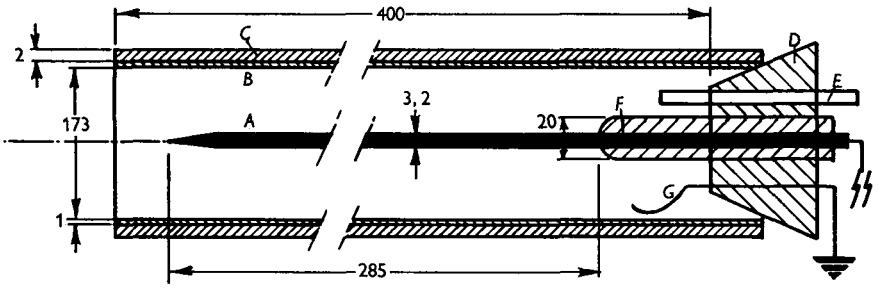

Fig. 1. Diagram of the collector tube of the electrostatic precipitator with agar collector. *A*, high-tension electrode; *B*, agar layer on earth potential; *C*, glass collector tube; *D*, rubber stopper; *E*, air outlet; *F*, insulator of high tension electrode (perspex); *G*, contact on earth potential; measurements are in mm.

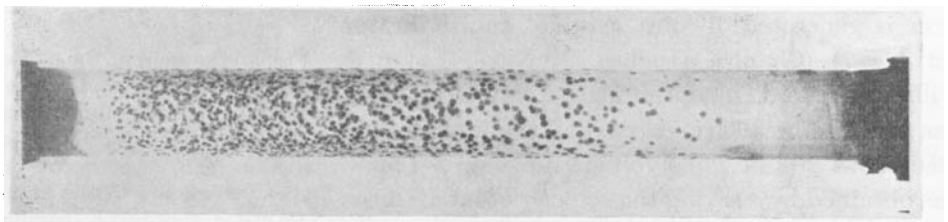

Fig. 2. Agar collector tube showing colonies of *Serratia marcescens* after electrostatic precipitation at 10 kV. and subsequent incubation at 25° C. for 30 hr.

The preparation of the agar tubes and operational details are described in the section dealing with the experiments.

The precipitator with water-film collector was constructed for continuous sampling of bacterial aerosols. This apparatus resembles the installation described by Whittet (1953). A film of water, flowing downwards over the inside wall of a vertical cylindrical tube forms the receiving electrode on earth potential. The centred wire is used as the positive electrode on high potential. At intervals, samples can be taken from the recirculating water. Fig. 3 shows a diagram of this type of electrostatic precipitator. Operational details are given below.

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Equation (5) provides a means of obtaining a fairly accurate estimation of the appropriate dimensions of a cylindrical electrostatic precipitator, assuming that size and charge of the aerosol particles are known. However, owing to lack of information on the electrical charges of ellipsoidal, bacterial aerosol particles in a positive corona discharge, such a calculation was useless. We have, therefore, tried out some collector tubes with single spore aerosols (see below) and finally adopted the tubes shown in Fig. 1 and Fig. 3.

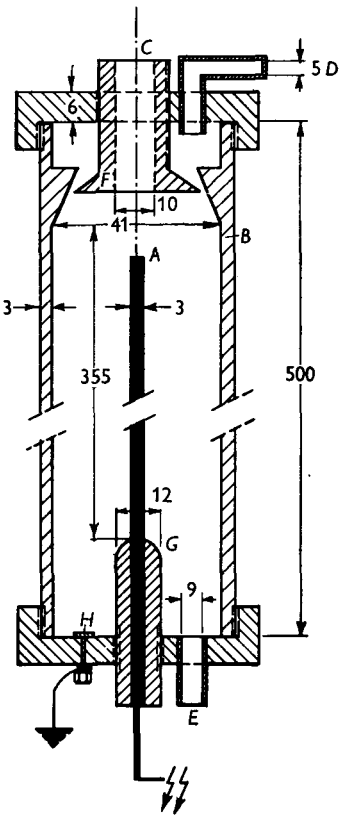

Fig. 3. Diagram of the collector tube of the electrostatic precipitator with water-film collector. *A*, high-tension electrode (stainless steel); *B*, collector tube (perspex or glass); *C*, air supply; *D*, water supply; *E*, combined water and air outlet; *F*, funnel for adjusting the water-film; *G*, perspex insulator of high tension electrode; *H*, contact on earth potential; measurements are in mm.

(2) *High tension generator*

To obtain positive high tension (*c.* 12 kV.) for the precipitator, a Philips line-output transformer type AT-2002 was used. This type was originally designed for a television set to generate the line deflexion current and the high tension for the picture tube. The circuit given by Jager (1953) was, with a few modifications, adopted here (Fig. 4).

Every time the current, which is normally flowing through the pentode (EL 81) and the primary coil of the transformer, is interrupted, a high tension peak is developed by the back e.m.f. on the secondary coil. This peak generates the precipitating tension after rectification. To obtain the periodical interruption of the current a switching wave-form is applied to the control grid of the pentode. This wave-form is generated by an 'astable' multivibrator (12 AU 7) (Chance, Hughes, McNicol, Sayre & Williams, 1949). The optimum switching frequency was found to be 8000 cyc./sec. and the interruption time about 20μsec. The voltage range of 2–12 kV. was obtained by varying the screen potential of the pentode by means of a potentiometer. This potential controls the anode current and consequently the back e.m.f. on the secondary coil. A current limiting resistor was interposed in the screen circuit to prevent the potentiometer from being overloaded. This limiting resistor also decreased the available output voltage from the possible maximum tension of 15 to 12 kV. which was still sufficient for our purpose.

The high internal resistance of the apparatus limits the maximum available current to a fraction of a milliampere and thus guarantees safe operation.

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PENETRATION TESTS

The collection efficiency (E) or the penetration ($1-E$) of a sampling apparatus may be determined by counting the numbers of particles precipitated in two identical samplers connected in series, or by measuring the concentration of an aerosol before and after passage through the sampler. The first method was used for the determination of the efficiency of the agar film precipitator; the electrostatic precipitator with water-film collector was tested with the slit sampler (Bourdillon, Lidwell & Thomas, 1941) by the second method mentioned. Since uniformity of aerosol particle size is essential for reliable results, the production of a suitable test aerosol will be discussed first.

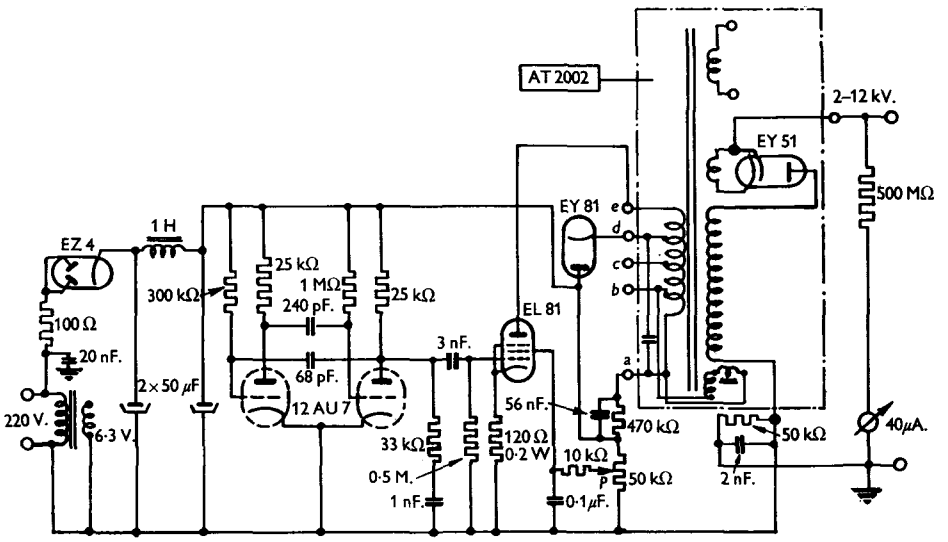

Fig. 4. Circuit of a generator for positive high tension from 2 to 10 kV.

(1) *Test aerosol*

In the penetration experiments, aerosols containing no more than one *Bacillus subtilis* spore per aerosol particle were used (single cell aerosols). These aerosols were generated by a modified Collison spray* at a constant air pressure of 1·2 atm. The droplets emerging from the spray were dried by a stream of filtered and dried air (relative humidity about 20 %) in a mixing chamber (*Mr* in Fig. 7). The dried spore cloud passes through a T tube to the sampling apparatus. A rotary pump maintains the air flow through the system. In our experiments the spore concentration varied from 1 to 10^5 spores/l. and the aerosol production from 10 to 50 l./min. The particle size distribution was determined by spraying a 5 % (w/w) eosine solution in distilled water and using a five-stage cascade impactor (Wilcox, 1953) constructed in this laboratory.

It will be seen from the data in Table 1 that 96 % of the droplets emerging from the spray have a diameter of 1·9μ or less (corresponding with a volume of about

* Kindly supplied by Dr D. W. Henderson, Microbiological Research Establishment, Ministry of Supply, Porton, Salisbury.

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4×10^{-12} ml. or less). Using homogeneous spore or cell suspensions of a concentration less than 10^9 spores (cells) per ml. in the Collison spray, only a negligible small number of droplets of the aerosol produced could contain more than one spore or cell. Spore suspensions were prepared by growing *Bacillus subtilis** in peptone water (1 %) containing 0·3 % glucose. Shake cultures were incubated at 30° C.

Table 1. *Determination of the particle size distribution of an eosine aerosol produced by the modified Collison spray*

Diameter* dried eosine particles, d in μ	Diameter† droplet D_r in μ	Cumulative percentage
0·38	1·03	43·5
0·48	1·30	75·9
0·68	1·85	96·1
0·97	2·61	97·5
1·35	3·67	99·8
2·70	7·32	100·1

* Microscopic counts on cascade impactor slides with special eyepiece graticule described by May (1945).

† Calculated droplet diameter $D_r = d\left(\frac{\rho E}{\rho W}\frac{1}{c_E}\right)^{\frac{1}{3}}$, where ρE is the specific gravity of the eosine solution of concentration c_E and ρW the specific gravity of water.

Sporulation was complete in 3–4 days, provided that proteose peptone (Gurr) was used; spore suspensions were prepared by suitable dilution of the cultures. Clump formation in the spray suspensions and sampling devices was prevented by the addition of 0·1 % (w/w) sodium alginate (Henderson, 1952).

(2) *Determination of the penetration*

According to Bourdillon *et al.* (1941), the penetration of a sampling apparatus, determined by connecting two samplers in series and counting the number of organisms collected in the first (N_1) and the second (N_2) sampler, can be expressed by

$$P = \frac{N_2}{N_1}. \tag{7}$$

This method gives accurate results provided that the penetration is less than 80 %, the samplers are identical and particle size is sufficiently uniform. When the penetration is determined by sampling the aerosol before and after passage through the sampler to be tested, the penetration can be calculated in the same way.

Combination of equations (6) and (7) gives

$$\log \frac{1}{P} = \log \frac{N_1}{N_2} = \frac{2wL}{2{\cdot}3Rv}. \tag{8}$$

This equation shows a linear relationship between $\log 1/P$ and L/v (L/v being the transit time of an aerosol through the precipitator). The penetration of both types

* Marburg strain, kindly supplied by the late Prof. Dr A. J. Kluyver, Laboratory of Microbiology, Technical University, Delft.

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of electrostatic precipitators has been measured at sampling rates ranging from 10 to 50 l./min. and at tensions varying from 4 to 10 kV. The results presented in Figs. 5 and 6 agree satisfactorily with the theoretical straightline relationship between log $1/P$ and L/v.

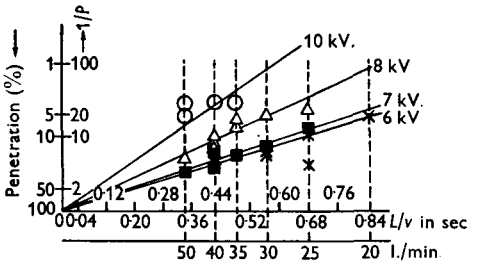

Fig. 5

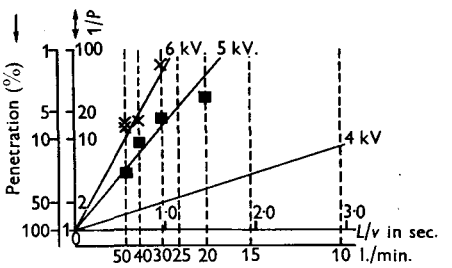

Fig. 6

Fig. 5. Penetration of the electrostatic precipitator with agar collector at different sampling rates and tensions.

Fig. 6. Penetration of the electrostatic precipitator with water-film collector at different sampling rates and tensions.

As can be concluded from Fig. 5, the precipitator with agar film collects aerosol particles with 90 % efficiency at a sampling rate of 25 l./min. and tensions varying between 6 and 10 kV. The efficiency of the precipitator with water-film collector at 5 kV. and 35 l./min. (Fig. 6) equals the efficiency of the agar collector at 8 kV. and the same sampling rate.

From equation (8) it follows that the slope of the log $1/P$ versus L/v lines, which can be expressed for a cylindrical precipitator by

$$\frac{2w}{2{\cdot}3R} = \frac{2eF}{2{\cdot}3R^2 \ln R/r 6\pi\eta a} n_s$$

is determined by the saturation charge n_s of the particles. Table 2 presents the particle charges calculated from the slopes of the lines in Figs. 5 and 6 (assuming that single spores are spherical particles with a diameter of $0{\cdot}8\mu$).

Table 2. *The electric charge of single spore aerosol particles of* Bacillus subtilis *spores calculated from penetration data (Figs. 5 and 6)*

Tension (kV.)	Slope of lines $\frac{\log 1/P}{L/v}$		Electric charge of aerosol particles (e.s.u. of charge)	
	Fig. 5	Fig. 6	In agar precipitator	In water-film precipitator
4	—	0·3	—	55
5	—	1·2	—	172
6	1·5	1·7	178	203
7	1·6	—	166	—
8	2·2	—	196	—
10	3·6	—	286	—

The data in Table 2 show that the electric charge of spore particles is fairly constant in the precipitators at tensions between 5 and 8 kV., suggesting that

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charging takes place by the ionic diffusion process. At 10 kV., however, the charge increases considerably, probably as a consequence of charging by ionic bombardment. The low charge obtained at 4 kV. is due to the poor ionization at that tension.

COMPARISON OF THE PRECIPITATOR WITH AGAR COLLECTOR WITH THE SLIT SAMPLER AND MEMBRANE FILTERS

The samplers were compared by determination of the viability factor, the reproducibility and the precision of the results. The viability factor is defined as the ratio of the numbers of viable bacteria collected in two parallel samplers (viable bacteria are those bacteria which grow out to visible colonies). The reproducibility follows from the results obtained on different days. The precision has been judged from the coefficient of variation.

(1) *Test circuit*

The air flow through the test system is shown in Fig. 7. The bacterial cloud produced by the modified Collison Spray (*Sp*) is mixed with filtered dry air (20–40 % relative humidity) in a mixing chamber (*Mr*). A circuit, following the principle of the Wheatstone bridge in electrical circuits and consisting of two parallel air resistors R_1 and R_2 (tubes of equal length and diameter) enables the

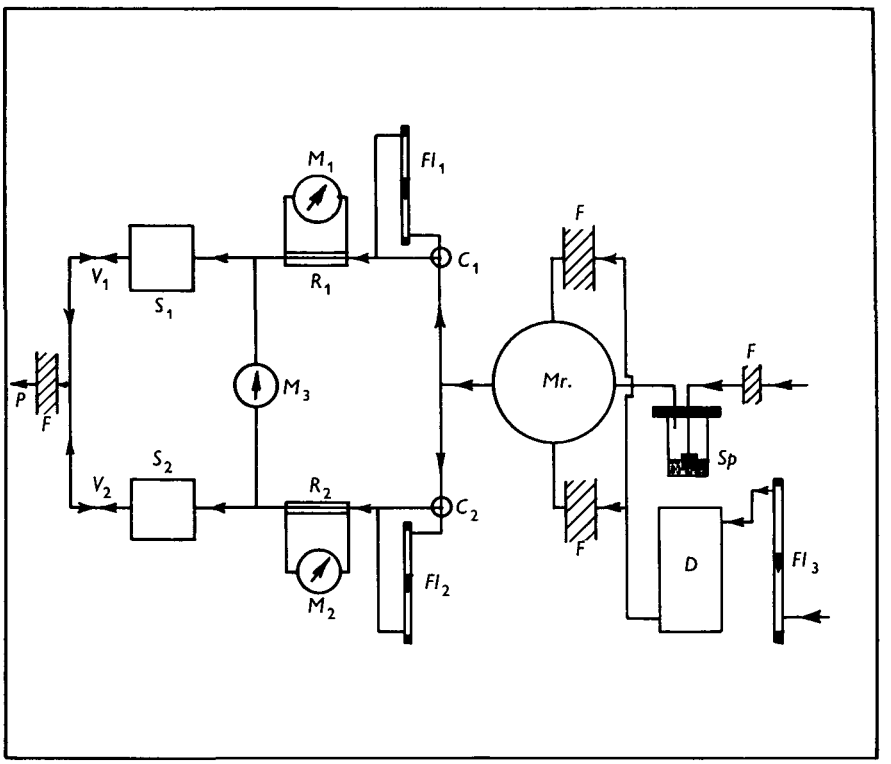

Fig. 7. Circuit for testing two samplers in parallel. *CA*, compressed air; *F*, filters; *Sp*, Collison spray; *Mr*, mixing chamber; *D*, silicagel drier; *Fl*, flowmeters; *C*, three way cocks; *R*, airflow resistors; *M*, manometers; *S*, samplers; *V*, regulating valves; *P*, vacuum pump.

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distribution of exactly the same volume of aerosol to each sampler by adjusting the manometer reading M_3 to zero with the regulation valve V_1 (or V_2). The air flow through the samplers is indicated by the flowmeters Fl_1 and Fl_2, which can be switched off during the sampling period with the three-way cocks C_1 and C_2. In most of the experiments, the air flow was for practical reasons kept at 17 l./min. through each sampler.

(2) *Test aerosols*

In addition to *B. subtilis* spore suspensions (see penetration tests) suspensions of *Bacterium coli* and *Serratia marcescens* were also used. These suspensions were prepared fromshake cultures in peptone broth (Bacto-peptone 1 %, glucose 1 %, NaCl 0·5 %, meat extract 0·3 %, pH 7·2). After incubation for 18 hr. at 30° C. the cultures were diluted with 0·1 % (w/w) sodium alginate.

(3) *Preparation of the agar collector*

The agar collectors were prepared by rotation (900 rotations/min) of a sterile glass tube filled with *c.* 60 ml. of molten agar (2·5 % in peptone broth) under simultaneous ice-cooling of the tube-wall. Air-borne contamination on cooling could be prevented by providing the tube with a perforated rubber stopper fitted with a small glass tube containing a cotton-wool plug. Before use the tubes were incubated overnight at 37° C. and 40–60 % relative humidity to dry the agar.

(4) *Slit sampler*

A Casella slit sampler (S. 2000, Casella, London) was used. The penetration was determined by sampling a test aerosol of *B. subtilis* spores before and after passage through the slit sampler with membrane filters and the electrostatic precipitator with agar collector. At a sampling rate of 17 l./min. the penetration was found to be *c.* 11 %. At this sampling rate the number of bacteria collected must therefore be corrected with a factor 100/89.

(5) *Membrane filters*

Two types of membrane filters were used, the Aerosol Assay filter (Milipore Filter Co, Watertown, Mass.) and the G-1 filter (Gruppe 1, 'rot gestempelt', Sartorius Werke, Göttingen). The penetration of these filters for *B. subtilis* spore aerosols, determined by mounting two filters of one type in series, was less than 3×10^{-5} % and independent of the sampling rate (10–30 l./min.). The filters were incubated in a Petri dish on filter paper saturated with peptone broth.

(6) *Viability factors and reproducibility*

A number of successive sampling tests* of 1 min. duration were carried out with two parallel samplers at one aerosol concentration and at a sampling rate of 17 l./min. in the test circuit outlined in Fig. 7. The mean viability factors (mean quotients of the numbers of viable bacteria collected) are given in Table 3 (electrostatic precipitator/slit sampler), Table 4 (electrostatic precipitator/membrane

* A number of successive 'tests' or 'a series of tests' will be indicated as 'one experiment'. An experiment is a series of tests performed on one day with one bacterial suspension.

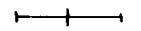
filter) and Table 5 (slit sampler/membrane filter). Since the distribution of the viability factors in a series of tests performed on one day was approximately normal, the 95 % confidence limits α_1 and α_2 of the mean viability factor were calculated with Student's table.

Table 3 shows that *c.* 35 % more viable *B. subtilis* spores were collected by the electrostatic precipitator than by the slit sampler. Viability factors obtained on sampling *Bact. coli* and *S. marcescens* at 10 kV. are significantly lower than at 7 kV. This is probably due to less ozone formation in the latter case. At 7 kV. about 25 % more viable *Bact. coli* cells were collected in the electrostatic precipitator; the number of *S. marcescens* cells was the same in both devices.

Table 3. *Mean viability factors obtained on sampling different bacterial aerosols with the electrostatic precipitator with agar collector and the slit sampler*

(Sampling rate: 17 l./min.; sampling period: 1 min.)

Date	Aerosol	No. of tests	Mean viability factors EP/SS (with 95 % confidence limits) 0·6 0·8 1·0 1·2 1·4 1·6 1·8
12. iv.	*Bacillus subtilis* spores	8	
4. iv.	*B. subtilis* spores	12	
12. v.	*Bacterium coli*	14	
23. iv.	*Bact. coli*	13	
19. iv.	*Bact. coli*	12	
13. v.	*Serratia marcescens*	14	
4. v.	*S. marcescens*	13	


experiments with electrostatic precipitator at 10 kV. and slit sampler.

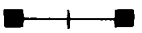

experiments with electrostatic precipitator at 7 kV. and slit sampler.

Table 4. *Mean viability factors obtained on sampling aerosols of* Bacillus subtilis *spores with the electrostatic precipitator with agar collector and membrane filters*

(Sampling rate: 17 l./min.; sampling period: 1 min.)

Date	Aerosol	No. of tests	Mean viability factors EP/membrane filters (with 95 % confidence limits) 1·0 2·0 3·0
6. iv.	*Bacillus subtilis* spores	6	
14. iv.	*B. subtilis* spores	5	

experiments with the electrostatic precipitator at 10 kV. and G–1 membrane filters of the Sartorius Werke (Göttingen).

From Tables 4 and 5, it appears that G-1 filters are unsuitable for analysing spore aerosols; Milipore filters yielded better results. This failure of the G-1 filters is probably caused by less favourable diffusion of nutrient broth through the filters on incubation.

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Table 5. *Mean viability factors obtained on sampling aerosols of* Bacillus subtilis *spores with the slit sampler and different membrane filters*

(Sampling rate: 17 l./min.; sampling period: 1 min.)

Date	Aerosol	No. of tests	Mean viability factor SS/membrane filters (with 95 % confidence limits)
			0·2 0·4 0·6 0·8 1·0 1·2 1·4 1·6 1·8 2·0 2·2
5. iv.	*Bacillus subtilis* spores	11	
13. iv.	*B. subtilis* spores	10	
25. iv.	*B. subtilis* spores	12	

⟝—+—⟞ experiments with slit sampler and membrane filters of Sartorius Werke (Göttingen).

●----+----● experiments with slit sampler and Aerosol Assay membrane filters Millipore Filter, Co., Watertown, Mass.).

Both types of filters gave highly unsatisfactory results with *Bact. coli* and *S. marcescens* aerosols since the number of viable cells collected was extremely low (less than 10 % of the slit sampler). These results have not been presented in Table 5.

The reproducibility of the viability factors listed in Tables 3, 4 and 5 obtained on different days was very satisfactory.

In some experiments, *Bact. coli* and *S. marcescens* aerosols, containing 2–3 cells/l. were sampled for 10 min. at a sampling rate of 17 l./min. The results are summarized in Table 6; they show that the viability factors agree with those found in the one minute sampling experiments of Table 3.

Table 6. *Mean viability factors obtained on sampling bacterial aerosols containing vegetative cells with the electrostatic precipitator with agar collector (at 7 kV.) and the slit sampler at a sampling rate of 17 l./min. for 10 min.*

Date	Aerosol	No. of tests	Mean viability factors EP/SS (with 95 % confidence limits)
			0·6 0·8 1·0 1·2 1·4
18. v.	*Bacterium coli*	3	
13. v.	*Serratia marcescens*	3	

(7) *Coefficient of variation*

For the comparison of the precision of the results a choice can be made between two indices of precision: the standard deviation s and the coefficient of variation c.v. (Stearman, 1955). The standard deviation is defined by

$$s = \left(\frac{\sum_{i=1}^{N} (x_i - \bar{x})^2}{(N-1)} \right)^{\frac{1}{2}} \qquad (9)$$

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(x_i = counts in individual tests; $\bar{x}$ = mean counts; N = number of tests in one experiment).

The C.V. is a measure of the amount of variation in terms of per cent of the mean,

$$\text{C.V.} = \frac{100s}{\bar{x}} \quad (s = \text{standard deviation.}) \tag{10}$$

The index which remains constant for each level of the mean seems to be the most suitable index of precision for the results obtained with the sampling device studied. In our experiments the standard deviation proved to be approximately proportional to the mean, thus indicating the independence between the C.V. and the mean. The C.V. was therefore chosen as index of precision. The C.V. was calculated for each series of N tests performed on one day (Tables 3–5). The mean $\overline{\text{C.V.}}$ from p experiments performed on p days is given by

$$\overline{\text{C.V.}} = \frac{\sum_{i=1}^{p} (N-1)(\text{C.V.})_i}{\sum_{i=1}^{p} (N-1)}, \tag{11}$$

where N = number of tests in one experiment and p = number of experiments.

The corresponding 95 % confidence limits α_1, and α_2 were calculated by means of the standard deviation of the C.V.

Table 7 records the mean C.V. and the 95 % confidence limits calculated from the results of Tables 3–5. With the exception of the G-1 filters, the precision of the different sampling apparatus is approximately the same.

Table 7. *Mean coefficients of variation of aerosol sampling experiments (Tables 3–5) with the electrostatic precipitator with agar collector, the slit sampler and two types of membrane filters*

(Sampling rate: 17 l/min; sampling period: 1 min.)

Sampling apparatus	No. of experiments	No. of tests	Mean coefficient of variation $\overline{\text{C.V.}}$ (%)	95 % confidence limits α_1	α_2
		Aerosols of *Bacillus subtilis* spores			
Electrostatic precipitator with agar collector (10 kV.)	4	31	10·0	8·5	11·4
Slit sampler	5	53	9·1	8·2	9·9
German G-1 membrane filter	4	32	20·3	18·0	22·6
American AA membrane filter	1	12	10·9	—	—
		Aerosols of *Bacterium coli*			
Electrostatic precipitator with agar collector (7 kV)	1	14	15·2	—	—
Slit sampler	1	14	12·0	—	—
		Aerosols of *Serratia marcescens*			
Electrostatic precipitator with agar collector (7 kV.)	1	14	11·3	—	—
Slit sampler	1	14	13·7	—	—

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COMPARISON OF THE ELECTROSTATIC PRECIPITATOR WITH WATER-FILM COLLECTOR WITH DIFFERENT IMPINGERS

The tests have been performed in the circuit outlined in Fig. 7. The aerosols mentioned before were used. The precipitator was compared with two types of impingers, the standard Porton impinger* and an impinger developed in this laboratory (MBL impinger).

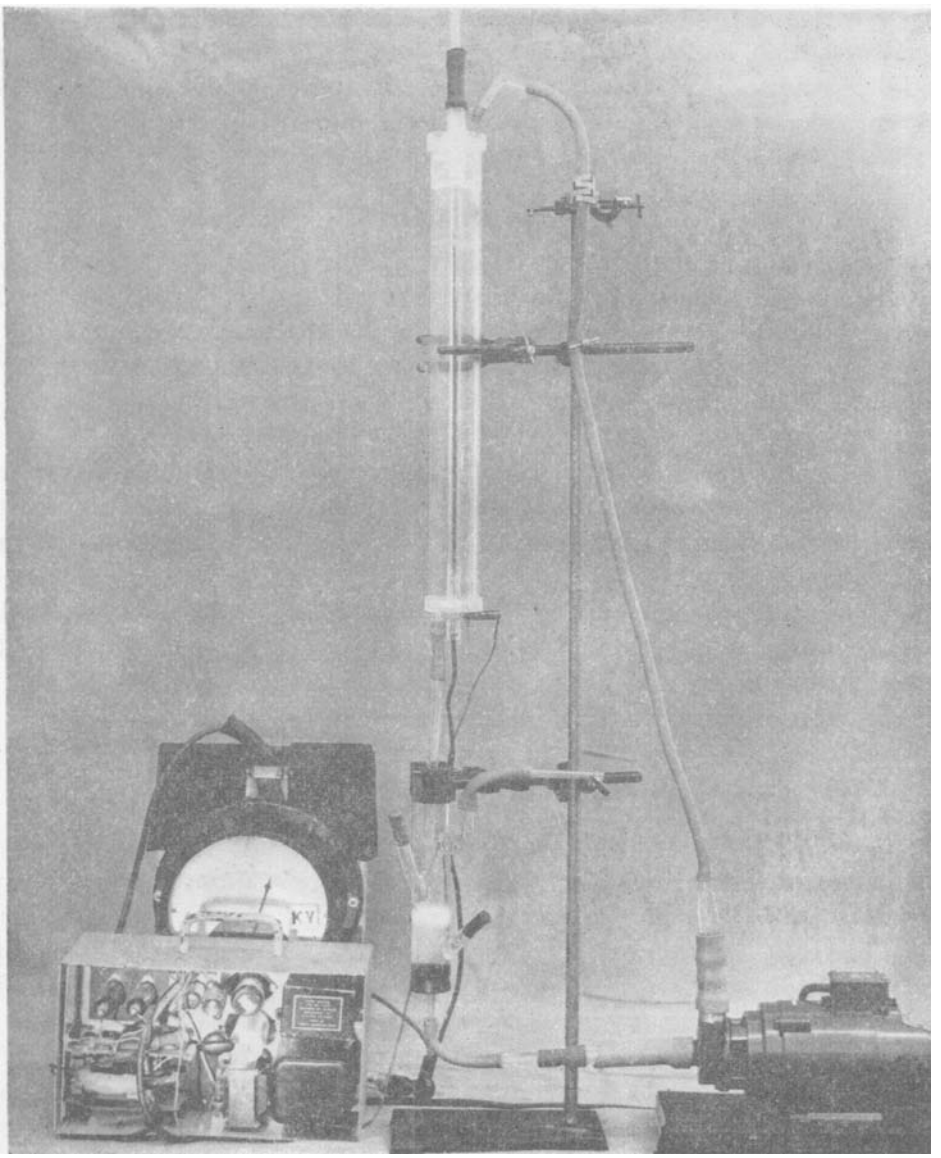


Fig. 8. Electrostatic precipitator with water-film collector, fluid container with sampling ports, circulation pump and high-tension generator.

* Kindly supplied by Dr D. W. Henderson, Microbiological Research Establishment. Ministry of Supply, Porton, Salisbury.

https://doi.org/10.1017/S002217240003744X Published online by Cambridge University Press

(1) *The electrostatic precipitator with water-film collector*

Fig. 8 gives a general view of the precipitator with accessories (cf. Fig. 3). The circulation pump employed was a Stuart centrifugal pump (type no. 12, 200 W.); the fluid displacement ranges from 1 to 10 l./min. Samples of the circulating fluid were taken from sampling ports. Rubber tubes were autoclaved for 20 min. at 120° C., the perspex tube and pump were cleaned by circulation of tap water. Sampling experiments were carried out at 11 l./min.; the penetration of the precipitator was found to be less than 1 % at 5–6 kV. (Fig. 6).

Special attention had to be paid to the central electrode. Iron and copper electrodes proved to be unsuitable. High quality stainless steel should be used to prevent corrosion which seems to increase ozone formation.

(2) *Impingers*

For a description of the Porton standard impinger we refer to the literature (Henderson, 1952). The penetration of single spore aerosols was determined by measuring the aerosol concentration before and after passage through the impinger by means of the slit sampler. At the maximal flow-rate (11 l./min.) the penetration was less than 1 %. The distance between the impinging jet and the base of the flask was 1·8 mm.

The MBL impinger was developed in this laboratory to meet the requirement of an apparatus sampling about 40 l./min. This all glass impinger holds an inlet tube (diameter 1·5 cm.) debouching into four holes of 1 mm. diameter at about 1 mm. from the flat bottom of the apparatus. The impinger contains 80 ml. of 0·1 % alginate solution. The penetration of single spore aerosols was found to be less than 1 % at a sampling rate of 40 l./min; at 11 l./min. the penetration increases to 13 %. Since the latter flow rate was chosen in the comparative experiments with the precipitator, the number of viable cells collected in the MBL impinger was corrected by a factor $\frac{100}{87}$.

(3) *Viability factors and reproducibility*

Single cell aerosols of *S. marcescens*, *Bact. coli* and *B. subtilis* spores were sampled by the electrostatic precipitator and the impingers for 5 min. at a flow rate of 11 l./min. The results are shown in Tables 8, 9 and 10. It appears from Table 8 that the precipitator is nearly equivalent to the Porton impinger in sampling spore aerosols.

The mean viability factor obtained on sampling *S. marcescens* aerosols, on the contrary, differs in most cases from unity. The viability of *S. marcescens* cells seems to be impaired by the precipitation process. The reproducibility of viability factors obtained on different days is satisfactory. *Bact. coli*, however, gave most irregular results.

A comparison of the precipitator with the MBL impinger illustrates that the reproducibility of the viability factors obtained on different days is poor (Table 9) Table 10 demonstrates that the two impingers are equivalent in sampling spore

Table 8. *Mean viability factors obtained on sampling aerosols of different organisms with the electrostatic film precipitator and the Porton standard impinger*

(Sampling rate: 11 l./min.; sampling period: 5 min.)

Date	Aerosol	No. of tests	Mean viability factor EP/Porton imp. (with 95 % confidence limits)
20. vii.	*Bacillus subtilis* spores	10	
22. viii.	*B. subtilis* spores	8	
26. vii.	*Serratia marcescens*	5	
23. viii.	*S. marcescens*	3	
8. xii.	*S. marcescens*	3	
9. xii.	*S. marcescens*	3	
9. xii.	*S. marcescens*	3	
21. vii.	*Bacterium coli*	5	
13. ix.	*Bact. coli*	13	

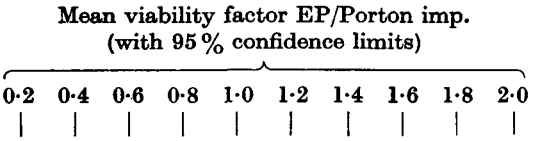


Table 9. *Mean viability factors obtained on sampling aerosols of different organisms with the electrostatic film precipitator and the MBL impinger*

(Sampling rate: 11 l./min.; sampling period: 5 min.)

Date	Aerosol	No. of tests	Mean viability factor EP/MBL imp. (with 95 % confidence limits)
27. vii.	*Bacillus subtilis* spores	7	
3. viii.	*B. subtilis* spores	10	
23. vii.	*Serratia marcescens*	7	
26. vii.	*S. marcescens*	8	
25. vii.	*Bacterium coli*	4	
14. ix.	*Bact. coli*	9	

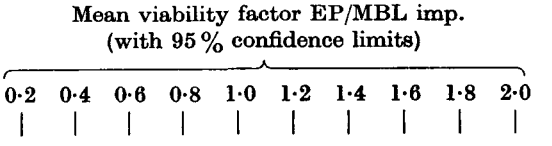
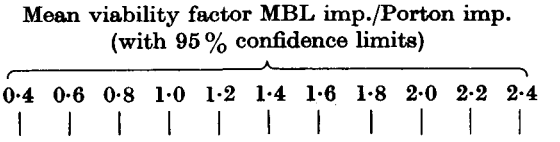


Table 10. *Mean viability factors obtained on sampling aerosols of different organisms with the MBL impinger and the Porton standard impinger*

(Sampling rate: 11 l./min.; sampling period: 5 min.)

Date	Aerosol	No. of tests	Mean viability factor MBL imp./Porton imp. (with 95 % confidence limits)
27. vii.	*Bacillus subtilis* spores	7	
1. viii.	*B. subtilis* spores	6	
26. vii.	*Serratia marcescens*	5	
25. viii.	*S. marcescens*	9	
25. vii.	*Bacterium coli*	4	
29. ix.	*Bact. coli*	8	



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aerosols. The reproducibility of the results with vegetative cells were rather disappointing.

The precipitator and the impingers were further tested in longer sampling periods up to 40 min. The equivalence of the apparatus for sampling spore aerosols is shown by the results in Table 11. Viability factors in *S. marcescens* experiments could be improved by replacing the alginate solution in the precipitator by 0·1 % peptone water; the reproducibility of the factors was satisfactory.

Table 11. *Sampling experiments for longer periods with the electrostatic precipitator with water-film collector and different impingers*

(Sampling rate: 11 l./min.; aerosol concentration: 2–3 cells/l.)

Date of experiments	Sampling time (min.)	No. of tests	Mean viability factor	Remarks
		Bacillus subtilis spore aerosols		
11. x.	5	3	EP/MBL imp. = 1·17	EP at 6 kV.; 0·1 %
11. x.	20	3	EP/MBL imp. = 1·26	alginate solution in
11. x.	40	4	EP/MBL imp. = 1·06	EP and impinger
26. ix.	5	2	EP/Porton imp. = 0·93	EP at 6 kV.; 0·1 %
26. ix.	20	2	EP/Porton imp. = 1·32	alginate in EP and
26. ix.	40	3	*EP/Porton imp.* = 1·07	*Porton impinger*
		Serratia marcescens aerosols		
12. x.	5	3	EP/Porton imp. = 1·06	EP at 5 kV.; 0·1 %
12. x.	20	2	EP/Porton imp. = 0·01	alginate in EP and
12. x.	40	4	EP/Porton imp. = 0·02	Porton impinger
9. xii.	5	8	EP/Porton imp. = 0·80	EP at 5 kV.; 0·1 %
21. xii.	20	5	EP/Porton imp. = 0·74	peptone water* in EP
20. xii.	40	5	EP/Porton imp. = 0·75	and Porton impinger

* Silicone antifoam used in these experiments.

(4) *Coefficient of variation*

The mean C.V. (see equation 11) and the 95 % confidence limits α_1 and α_2 calculated from the data in Tables 8–10 are presented in Table 12. The precision of the electrostatic precipitator and the impingers on sampling spore aerosols is about the same. With *Bact. coli*, and *S. marcescens* aerosols, the precision was unsatisfactory.

DISCUSSION

The electrostatic precipitators described in this paper have been compared with the slit sampler, membrane filters and impingers. Three criteria were used for the evaluation of the apparatus, viz. collection efficiency, survival of the organisms collected and precision of the results.

The results of penetration tests demonstrate that a collection efficiency of 90 % or more (penetration of 10 % or less) can easily be obtained by both types of precipitators.

The survival of organisms has been expressed by the viability factor. The data concerning this factor point to a rather favourable position of the agar collector. The membrane filters proved to be unsuitable for sampling vegetative organisms.

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The results obtained with the precipitator with water-film collector and impingers were less satisfactory. Variations between the viability factors obtained on different days occurred. The equivalence of the precipitator with water-film collector and the Porton impinger for sampling spore aerosols could be demonstrated. With *S. marcescens* aerosols there was a loss of *c.* 25 % of the viable cells in the precipitator.

Table 12. *Mean coefficients of variation of aerosol sampling experiments with the electrostatic precipitator with water-film collector and two types of impinger (Tables* 8–10)

(Sampling rate: 11 l./min.; sampling period: 5 min.)

Sampling apparatus	No. of experiments	No. of tests	Mean coefficient of variation $\overline{\text{c.v.}}$ (%)	95 % confidence limits αx_1	95 % confidence limits αx_2
Bacillus subtilis spore aerosols					
Electrostatic precipitator (6 kV.)	4	35	11·3	10·5	12·1
Porton standard impinger	3	24	10·2	9·0	11·5
MBL impinger	4	28	12·1	10·9	13·3
Bacterium coli aerosols					
Electrostatic precipitator (6 kV.)	3	18	18·8	16·4	21·2
Porton standard impinger	4	30	9·6	8·7	10·5
MBL impinger	4	25	16·9	15·8	18·0
Serratia marcescens aerosols					
Electrostatic precipitator (5 kV.)	5	27	17·5	16·4	18·6
Porton standard impinger	3	17	24·8	21·8	27·8
MBL impinger	2	16	14·1	12·4	15·8

The coefficients of variation, indicating the precision of the apparatus, varied with the type of aerosol. With spore aerosols coefficients of about 10 % were obtained; the precision with aerosols of vegetative organisms was considerably less for the precipitator as well as for other sampling apparatus. This contrasts with the results obtained by Henderson (1952) who reported a coefficient of variation of about 6 % for *B. subtilis* spores as well as *S. marcescens* aerosols sampled with the Porton impinger. The differences may be due to differences of bacterial strains or cultivation methods. Henderson used cells obtained from the surface of solid media whereas we used shake cultures.

From a practical point of view the electrostatic precipitators have the advantage of a low air resistance. At the same collection efficiency the resistance of the precipitators is more than ten times less than the resistance of the impingers, slit sampler and membrane filters. Therefore, the electrostatic precipitators can be provided with a simple fan, whereas the other samplers need a vaccum pump. The preparation of agar tube collectors and the reconstruction of a water-film collector are, on the other hand, rather complicated.

SUMMARY

1. The electrostatic precipitation of single cell bacterial aerosols has been studied. Two types of electrostatic precipitators of the wire-in-tube construction have been developed. In one type the collector is a tube with nutrient agar, in the other a vertical water-film.

2. Collection efficiencies of 90 % and more could be obtained at sampling rates of 25 l./min. and tensions varying from 5 to 10 kV.

3. The survival of organisms in electrostatic precipitators has been measured in comparison with other samplers, viz. the slit sampler, membrane filters and impingers. The equivalence of the electrostatic precipitators and other samplers in sampling spore aerosols could be demonstrated. Aerosols containing vegetative organisms were sampled equally well in the agar tube precipitator as in the slit sampler. In the precipitator with water-film collector there is a loss of *c.* 25 % of viable cells.

4. The practical advantage of the electrostatic precipitators is their very low air resistance.

The authors feel greatly indebted to Prof. Dr J. A. Cohen and Dr F. Wensinck for their helpful criticism of the manuscript, to Mr W. de Vries who designed and constructed the generator for extremely high tension; to Mr R. van Strik, Philips-Roxane (Weesp, Netherlands), for statistical advice and computations and to Miss H. Kranenburg for technical assistance.

LIST OF SYMBOLS USED IN THE TEXT

a	radius aerosol particle (cm.)
c	concentration aerosol (number of particles/ml.)
c_0	concentration aerosol at entrance of collector
c_L	concentration aerosol at distance L from entrance of collector
c_E	concentration eosine solution (g./g.)
C	$(\overline{v^2_{\text{ions}}})^{\frac{1}{2}}$ from kinetic gas theory (cm./sec.)
c.v.	coefficient of variation
D	dielectric constant
D_r	droplet diameter (μ)
d	diameter of a dried aerosol particle (μ)
E	efficiency of electrostatic precipitation
E_0	field strength (kV./cm. or e.s.u. of pot./cm.)
e	electronic charge ($4{\cdot}8 \times 10^{-10}$ e.s.u. of charge)
F	potential difference (kV. or e.s.u. of pot.)
k	Boltzmann constant (erg/° C.)
L	length of electrostatic precipitator (cm.)
l.	litre
N	number of tests in one experiment on one day
N_0	ion concentration (number of ions/ml.)
n	charge of an electric charged aerosol particle (e.s.u. of charge)

n_s saturation charge of an electric charged aerosol particle (e.s.u. of charge)
P penetration of an electrostatic precipitator
R radius collector cylindrical electrostatic precipitator (cm.)
r radius wire electrode in a cylindrical electrostatic precipitator (cm.)
s standard deviation
T absolute temperature (° K.)
t time (sec.)
V viability factor
v linear velocity (cm./sec.)
w precipitation velocity of an aerosol particle (cm./sec.)
$\alpha_{1,2}$ 95 % confidence limits
η viscosity of air ($= 1{\cdot}82 \times 10^{-4}$ poise at 18° C.)
ρ_E specific gravity eosine solution of concentration c_E
ρ_W specific gravity water

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(*MS. received for publication* 24. I. 57)