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Thermal Precipitation for Sampling Air-borne Microorganisms[1]

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For several years the applications of thermal precipitation have been investigated at the Georgia Institute of Technology (Kethley *et al.*, 1952; Gordon, 1953, 1954; and Gordon and Orr, 1954). Some of the recent investigations have been concerned with thermal precipitation as a means for the collection of viable air-borne microorganisms. Information on this work is being presented because the method, which might seem unlikely since heat is required, actually gave satisfactory results. The technic evolved, the tests made, and the results obtained are included.

MATERIALS AND METHODS

One type of thermal precipitator was used throughout the study, a model of the device described by Kethley *et al.* (1952) which is now manufactured and distributed by the Roy A. Martin Co., Atlanta, Georgia, under the trade-mark name Thermopositor. In this precipitator the air to be sampled enters at the center of a heated surface and flows radially outward between the heated surface and a cooled surface separated only 0.015 in. Any particle matter suspended in the air passing into the thermal field is forced onto the cooled surface or onto such substrate as may cover the cooled surface. Figure 1 shows the device with the collecting chamber open so that the heated and cooled surfaces may be seen.

In this study, the thermal precipitator was operated at a sampling rate of 0.3 L per min with the heated surface at 125 C and the cooled surface at the tap water temperature, approx 25 C. Sampling times varied between 15 sec and 5 min. Ordinary filter paper (Whatman No. 1, for example) saturated with a nutrient solution was found to be quite satisfactory as the substrate onto which the microorganisms were collected. As standard practice, each filter paper was first dipped into a solution containing 1.15 per cent nutrient agar (Difco) and 0.8 per cent brain-heart infusion (Difco). The paper was stored in a covered dish until used or transferred directly (after excess liquid had dripped off) with sterilized tweezers to the cooled plate of the precipitator. When the paper was located, the heated plate of the precipitator was put into place, and sampling was begun.

After the desired volume of air was sampled, the filter paper with its deposit of microorganisms was placed, with the deposit up, on a regular nutrient-agar surface in a Petri dish. The dish was finally inverted and the deposited microorganisms incubated at 37 C. Figure 2 shows filter papers after deposits of *Serratia marcescens* and *Bacillus subtilis* had been incubated for 17 hr and then dried. The clear space around the edge is indicative that all microorganisms in the air sampled have been collected. The incoming air, it will be remembered, enters at the center of the collecting surface and flows outward in all directions. If the heat intensity and the flow rate are properly adjusted, suspended matter is deposited before the edge of the collecting surface is attained.

Washed and dried cells of *S. marcescens*, strain 8 UK (Chemical Corps), and *B. subtilis* spores (Chemical Corps) were suspended in distilled, de-ionized water and were put separately into the air of a test chamber using a DeVilbiss No. 40 atomizer arranged to operate continuously at 10 psig air pressure. The resulting aerosol was stirred slowly. The humidity in the chamber was regulated by passing all or part of the inlet air through freeze-out traps if lowered humidities were desired or over heated water containers if higher humid-

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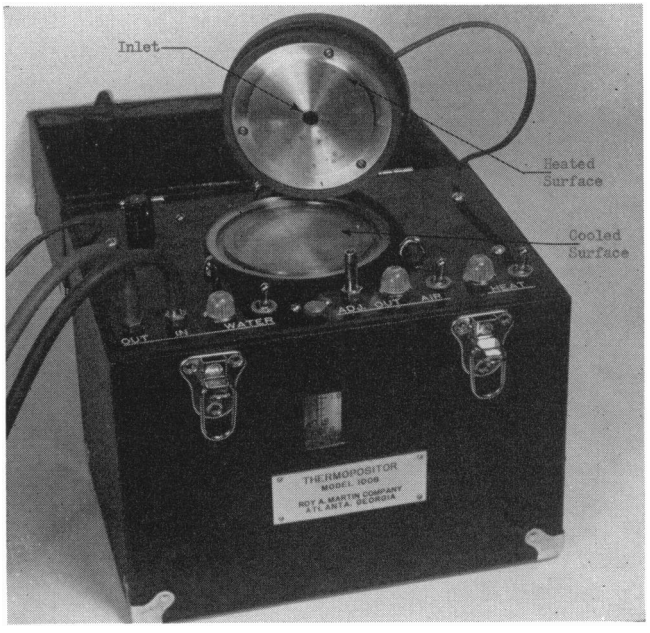

FIG. 1. Thermal precipitator

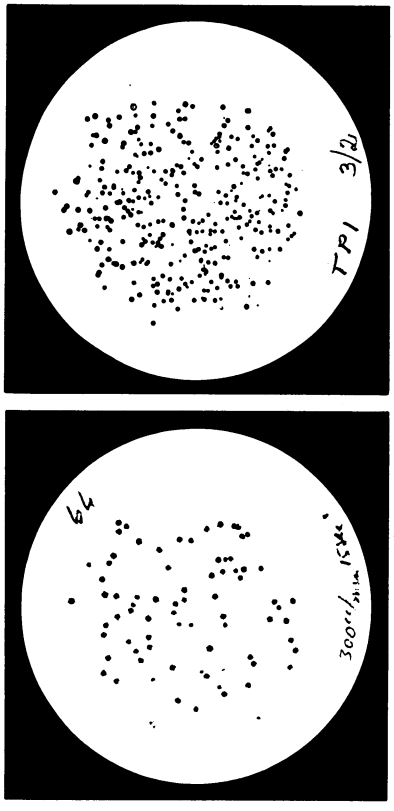

FIG. 2. Typical filter paper deposits of microorganisms after culturing. Above: *Serratia marcescens*; below: *Bacillus subtilis*.

ities were desired. The temperature in the chamber was likewise regulated by heating or cooling as required. The humidity of the air in the chamber was indicated by a lithium chloride cell. Along with other tests reported by Orr and Kordecki (1955), the aerosols, as set up in the test chamber, were sampled with the thermal precipitator, a liquid midget impinger and a liquid critical-orifice impinger. The various samples were collected as nearly simultaneously as possible.

The midget impinger employed was designed for dust collection. It had a 0.1-cm orifice and was operated at a prescribed rate of 2.8 L per min in this study. Samples were collected in 1 to 5 min in 10 ml of 0.3 per cent beef broth. This liquid was then diluted, portions of it were

TABLE 1. *Concentration of viable air-borne cells as determined from thermal precipitator and impinger samples*

Relative Humidity*	Viable Cells Collected By		
	Thermal precipitation	Midget impinger	Critical-orifice impinger
	Serratia marcescens		
%	*cells/L*	*cells/L*	*cells/L*
3	53	27	75
5	123	1	1†
7	55	36	—
8	333	161	—
12	750	607	—
13	243	268	411
15	267	143	—
21	4,880	3,210	—
22	1,150	714	—
22	1,290	1,320	—
22	130	52	150
22	2,790	1,650	—
23	21	26	19
26	2,980	2,140	—
28	1,973	1,560	1,740
33	3,393	—	1,910
35	700	54	—
37	52	29	69
38	142	36	89
38	1,270	3,560	—
39	6,300	3,270	7,030
40	7,170	7,710	—
49	770	714	—
56	4,670	1,940	4,940
67	6,900	8,760	—
78	753	660	—
79	1,300	1,770	—
99	13,000	18,000	—
	Bacillus subtilis		
25	17,800	—	14,700
27	11,200	—	7,850
45	1,130	—	1,180
45	5,000	—	4,300
47	18,900	—	53,300
48	4,190	—	3,740
49	18,300	—	16,200
50	25,300	—	14,600
60	369	258	411
65	1,750	2,690	2,480
78	38,900	—	143,000
78	33,600	—	11,200
79	13,500	—	17,900

* Dry bulb temperatures ranged between 18.33 and 41.67 C (65 and 107 F).

† No tests.

plated in triplicate using 2.3 per cent nutrient agar, and the plates were incubated. A few drops of mineral oil were added to the beef broth to prevent excessive bubbling.

The critical-orifice impinger operating at its critical velocity had a flow rate of 1.07 L per min. As with the midget impinger, 0.3 per cent beef broth was used, samples were plated in triplicate, and mineral oil was added to prevent excessive bubbling. Sampling times varied from 1 to 10 min.

The colonies on each plate were counted and the data were converted to a common basis, the concentration of viable cells in the initial aerosol.

RESULTS

A comparison of sampling results for various humidities and aerosol concentrations is presented in table 1. Of the 29 tests in which the thermal precipitator can be compared with the midget impinger, the precipitator results indicated a greater aerosol concentration in 20 tests. If one omits the test in which the thermal precipitator gave many times the concentration indicated by the midget impinger, the ratio between the aerosol concentration from thermal precipitator data and midget impinger data indicates a median value of 1.39. On the same basis, the average value of the ratio is 1.86. The thermal precipitator seems to have provided more complete sampling than the midget impinger.

The 24 tests comparing the thermal precipitator with the critical-orifice impinger resulted in 12 tests in which the thermal precipitator indicated the greater aerosol concentration and 12 tests in which the critical-orifice impinger indicated the greater concentration. Again neglecting the test in which the thermal precipitation result was excessive, the median value of the ratio between thermal precipitation and the critical-orifice impinger results was 0.967; the average value was 1.09. The thermal precipitator, when operated properly, is claimed 100 per cent efficient for particle matter resolvable by the electron microscope. Other investigations (Silverman, 1954) indicate the critical-orifice impinger to be essentially 100 per cent efficient. The present work certainly indicates them to be comparable.

Other tests of the efficiency of thermal precipitation for collecting viable microorganisms were made. When the rate of aerosol generation, the viable content of the cell suspensions, the expected die-away rate, and losses due to aggregation and collection on walls and so forth were taken into account, complete viable cell collection was indicated. The second of two precipitators operated in series collected no microorganisms, showing again complete collection in one precipitator.

Certain advantages were found in thermal precipitation. Using the technic outlined, sampling is rapid, simple and direct. The resulting colonies are on the surface and not embedded in the substrate. The substrate may be handled easily and dried for storage if desired. Direct thermal precipitation eliminates the dilution of the collected sample which is necessary with impinger technics. For relatively low cell concentrations this can be quite advantageous. By the same token, when the cell concentration is high, direct thermal precipitation can result in too many cells for satisfactory culturing and counting. This effect probably accounts for some of the low values obtained by thermal precipitation at high aerosol concentrations. One difficulty was noted. When the sampling period exceeds about 5 min and the sampled air is relatively dry, a drying of the filter paper substrate occurs, beginning at the center, the air inlet point, and gradually spreading outward. With microorganisms such as *S. marcescens* the dried spot is usually devoid of colonies. As is sometimes done with filters to maintain high humidities when microorganisms are collected from the air, the inlet air might be humidified and this difficulty overcome. This possibility was not tried; the results given in table 1 are all for sampling periods of 5-min duration or less.

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SUMMARY

Thermal precipitation using a newly developed instrument is compared with liquid impingement technics for the collection of viable, air-borne microorganisms. The technic is described, and results for a variety of humidity conditions using prepared aerosols of *Serratia marcescens* and *Bacillus subtilis* are given. It is concluded that thermal precipitation provides a valuable new collection method.

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