

# THE COLLECTING OF AIRBORNE MICROORGANISMS

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During an investigation of the effects of two standard humidity control systems on airborne microorganisms an extensive study was made of the techniques for collecting airborne microorganisms from air streams and ventilated rooms. The results of the studies on collecting techniques as well as the data obtained for the two air conditioning facilities are presented herein.

The purpose of studying two different types of humidity control systems, namely Kathabar and a conventional system using refrigeration coils was to compare the effects of the two systems on the number of airborne microorganisms in treated areas. In the Kathabar, cooling coils are always dry. In conventional refrigeration systems the coils are wet when the air is being cooled but dry off when the refrigeration is shut off. The refrigeration coils are alternately wet and dry as the refrigeration compressor cycles on and off. Since bacteria and molds grow readily in moisture a study of the effect of drying off of the refrigerator coils was included as a part of this study.

A search has been underway for many years to find an ideal system of controlling airborne microorganisms in single rooms and buildings. The requirements for characterizing air sanitizing agents have been discussed by Kuehner (1952). Several systems have already been devised for removing airborne microorganisms, (Jennings, 1948; Berry, 1941) but the problems of control in occupied spaces is complicated by the fact that humans release microorganisms from the skin, hair, clothing and mucous membranes. The problem is to reduce "the number of pathogenic organisms in a given space to such a small amount that the chance of cross-infection becomes remote," (Jennings, 1949).

One of the factors in the control of airborne microorganisms is the control of humidity to reduce the growth of organisms on room surfaces and fixtures, (Nagy *et al.* 1954). The control of humidity is also important from the point of view of human comfort and productivity.

There are many industrial applications in which the control of both humidity and microorganisms is important, such as in the manufacture of foods, pharmaceuticals and beverages, (Lund, 1948; Shimwell, 1949). Both are important also in hospitals and operating rooms for the control of static electricity and cross infection, (Smith and Loosli, 1952). In research laboratories where disease-producing organisms are being utilized, the control of airborne microorganisms is absolutely essential, (Decker *et al.* 1954).

The evaluation of control systems can only be made if reliable and accurate methods of collection have been worked out.

## MATERIALS AND METHODS

### *Collecting Criterion*

During the preparation for these experiments it was realized that methods of sampling the air stream would have to be devised which would give a true sample of the air and hence, reliable and consistent data.

Three techniques were selected which were thought to meet these requirements. They have been designated the all-glass impinger technique, the Electrostatic Bacterial Air Sampler and the funnel impinger technique. The open Petri dish technique was used in the rooms being studied for comparison only.

*Collecting with the all-glass impinger*

One system of collecting samples consisted of all-glass impingers<sup>1</sup> connected to a Central Scientific "Hypervac" vacuum pump. The all-glass impinger had a critical orifice sized for 13.3 liters of air per min. so that the air flow was constant as long as the vacuum on the outlet of the impinger was 16.5 in. Hg or more. It is designed to collect organisms less than 20  $\mu$  in size. The sample tubes ranged from 6 mm. to 10 mm. inside diameter and were sized so that the sample velocity was approximately that of the air in the duct.

Sample tubes with 3 in. radius right angle bends were inserted through holes in the side of a duct, with a 4 in. leg pointed into the air stream. The other end of the sample tube was connected to the inlet of the all-glass impinger with intravenous rubber tubing. The outlet of the all-glass impinger was connected by vacuum tubing, copper tubing and a valve to a manifold, which was in turn connected to the vacuum pump. The vacuum pump had a capacity sufficient to maintain a vacuum of more than 16.5 in. Hg while pulling through 5 impingers. The test procedure was to install the sample tubes and impingers at as many as 5 stations and then start the vacuum pump. The air samples totaling 10 cu. ft. of air were bubbled through the impingers simultaneously for 21.2 min. At the end of this time, the pump was stopped, and the impingers were removed and stoppered with sterile cotton.

Previous to the taking of air samples, 25cc. of isotonic saline solution was placed in each of the impingers, and the impingers sealed, plugged with cotton and sterilized. Some loss of water occurred during the taking of the sample because of the lower humidity of the air flowing through the impinger. However, the final volume was used in calculating the concentration of microorganisms.

After the collection of air samples the impingers were again taken to the laboratory where the quantitative evaluation of microorganisms were performed. Aliquot portions were placed in sterile Petri dishes and mixed with freshly prepared Tryptose Blood Agar. After cooling, the petri dishes were inverted and incubated at 25°C and 37°C for 48 to 96 hr. The resultant colony growth was observed and counted. The tabulation was computed upon the aliquot portion and the average colony count.

*Collecting with the Electrostatic Bacterial Air-Sampler*

A Luckiesh-Holladay-Taylor (1946) Electrostatic Bacterial Air-Sampler duplex type, manufactured by the General Electric Company, was also used in the evaluation of air-borne microorganisms.

The sampler was connected to the air ducts by means of tubing and a funnel, and a total of 10 cu. ft. of air passed through the sampler.

Samples as taken from the sampler were then incubated 48 to 96 hr. at 25°C and 37°C and the colony counts recorded.

*Collecting with Funnel Impinger*

The funnel impinger method utilizes the impingement principle directly upon the surface of nutrient agar, Hollaender and Dalla Valle (1939). The air enters through the stem of a 45° glass funnel whose open end diameter is slightly smaller than that of a standard petri dish. The open end of the funnel was raised 3 mm. above the surface of the agar to permit the escape of air. The rate of air flow was metered at 1 cu. ft. per min. for 10 min.

The petri dishes containing Tryptose Blood Agar were then incubated at 25°C and 37°C for 48 to 96 hr., and the colony counts recorded.

<sup>1</sup>Manufactured by the Ace Glass Co. Vineland, New Jersey.

### Collecting with the Open Petri Dish

The "open dish" method is based on the impingement principle, wherein the heavier particles of dust, droplets and moisture to which microorganisms may be attached settle upon the surface layer of the nutrient agar. Freshly prepared Tryptose Blood Agar petri dishes were opened and exposed for fifteen minutes in the room air serviced by the air conditioning equipment. The petri dishes were then incubated at 25°C and 37°C for 48 to 96 hr. and the colony counts recorded.

### Air Conditioning Equipment and Collecting Stations

Actual installations in two hotels in Toledo have been used for the experimental work. One hotel has a Kathabar installation for the control of humidity. The other hotel has refrigeration coils for the control of humidity in circulating air.

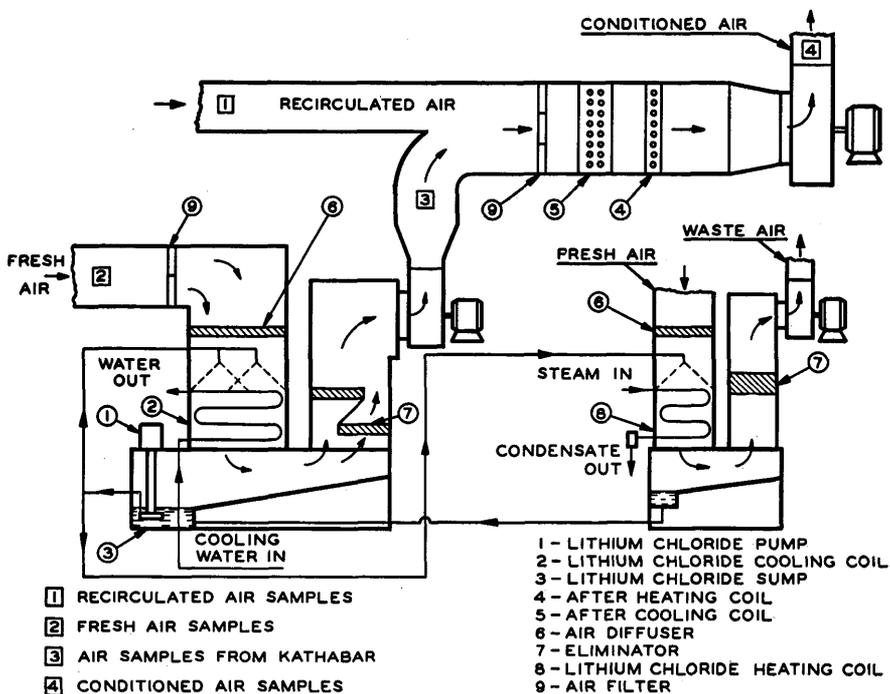


FIGURE 1. Kathabar equipment used in the experimental work.

The Kathabar system of humidity conditioning is a mechanical unit which brings air into contact with a lithium chloride brine, this solution removing or adding moisture in selective amounts. The operation is continuous and under automatic control.

Lithium chloride has a great moisture absorptive capacity, the amount of moisture removal from the air depending on the temperature of the brine. Regeneration of the solution is accomplished by heating to drive off the moisture absorbed, increasing the concentration of the solution.

Air conditioning equipment in hotel "A" consisting of a recirculated air system with fresh air make-up is diagrammed in figure 1. Fresh air is pulled through a bank of filters (9) thence through an air diffuser into a water cooled spray of lithium chloride solution. (1) (2) (3). Lithium chloride is removed from the air by an

eliminator (7). The air is then delivered to a plenum where it is mixed with the recirculated air from the room. The mixed air is then pulled by the main fan through a bank of filters, a direct expansion cooling coil (5) an after-heat coil (4) and delivered to the room. The lithium chloride regenerating equipment is shown on the right (6) (7) and (8).

Air volumes handled were 4,150 cfm of fresh air, and 5,600 cfm of recirculated air for a total circulation of 9,750 cfm.

Air samples were taken from the fresh air duct to the Kathabar, the duct leaving the Kathabar, the recirculated air duct, and the plenum after the heating coil as indicated on figure 1.

Air conditioning equipment in hotel "B" consisted of a recirculated air system with fresh air make-up essentially the same as in hotel "A" except that the fresh air was untreated. In this installation, the only fan was the main air fan, the proportion of fresh air to recirculated air being controlled by dampers. The fresh air was pulled through a door into a corridor, through filters in the corridor wall and into the equipment room which served as a fresh air plenum. It then entered a grille into the fresh air duct which terminated in a plenum where it mixed with the recirculated air. The mixed air then entered a conventional refrigerated air conditioning system. From this unit it entered the fan and was discharged into the room supply duct.

Air volumes handled were 1,735 cfm of fresh air and 2,685 cfm of recirculated air for a total circulation of 4,420 cfm.

Air samples in hotel "B" were taken from the fresh air duct, the return air duct, the mixed air plenum, the room supply duct.

#### RESULTS

The results of the samples of airborne microorganisms collected by three different techniques are presented in table 1. All samples were collected at the recirculated air duct of the Kathabar installation in hotel "A".

TABLE I  
*Comparison of Sampling Methods*

	ALL GLASS IMPINGER	ELECTRO-STATIC AIR-SAMPLER	FUNNEL IMPINGER
Number of samples taken	23	36	19
Average no. of colonies 10 <sup>3</sup> of air	224	221	26

These data indicate that the electro-static air sampler and the all glass impinger are about equally effective for the collection of air borne microorganisms but that the funnel impinger collects only about 11.5 percent as many organisms as do the other techniques.

During the period when other sampling methods were being checked in the recirculated air duct, samples were collected simultaneously by the open petri dish method from the room being treated. Of twenty-one samples taken during exposures of 15 min. each, the average number of microorganisms collected was 23. There can be no direct comparison because the volume of air from which the fall-out of microorganisms occurred is not known and the time of exposure was shorter than the exposure time by other methods. However the number collected was roughly 10 percent of the number collected by the all glass impinger and the electro-static sampler in 10 cu. ft. of air. This indicates that the open petri dish technique is not adequate for quantitative measurements.

In view of the larger number of microorganisms collected by the all glass impinger all future experiments were with that equipment.

The next experiment was concerned with the number of microorganisms collected from two stations at hotel "A" designated as station 2 and 3 on figure 1. Thirty four samples were collected simultaneously at each station and the data are reported in table 2.

TABLE 2  
*Air Samples of Microorganisms Taken from Ducts at Hotel "A"*

	Air to Kathabar Station 2 (fig. 1)	Air From Kathabar Station 3 (fig. 1)
Number of Samples Taken	34	34
Average no. of colonies 10 ft. <sup>3</sup>	525	9.7

Of the microorganisms entering the Kathabar only 1.8 percent remained in the air that leaves. 98.2 percent of the microorganisms were removed.

In the previous experiment the samples were collected only at the entrance and exit to the Kathabar. However, since only the fresh air is passed through the Kathabar a further check was made on the recirculated air and the mixture of recirculated air and Kathabar treated air, to determine whether the number of microorganisms is reduced in the air returned to the room as would be expected. Twenty-one samples were again taken simultaneously at four stations. The results are reported in table 3.

TABLE 3  
*Average Number of Airborne Microorganisms Collected at Four Stations Simultaneously. All Numbers Represent the Count for 10 Cu. Ft. of Air.*

	Recirculated air Station 1 (fig. 1)	Fresh air to Kathabar Station 2 (fig. 1)	Air from Kathabar Station 3 (fig. 1)	Conditioned air after fan Station 4 (fig. 1)
Number of samples taken	21	21	21	21
Average number of colonies	223	220	1	119

These results show that the number of airborne microorganisms in the air returned to the room was reduced to approximately one half of the original room air count because the Kathabar had removed the airborne microorganisms.

Previous experiments had been concerned with total microorganisms which occurred in the sampling areas. Since the Kathabar proved to be an effective agent in removing microorganisms from the air, the effect of the equipment on removing pathogenic forms was also determined. Pathogens identified were of the following two types: Staphylococcus aureus and Streptococcus B hemolyticus (group A). The average counts obtained from 21 samples collected simultaneously at four stations are reported in table 4.

As explained earlier the main feature of Hotel "B" is dehumidification of room air by refrigerating the air, condensing out moisture on the cooling coils.

In Hotel "B" samples were collected from the air ducts while the refrigeration was on. In addition the refrigeration was turned off for as long as 1½ hr. to

collect samples at intervals in order to make counts of microorganisms from the air passing through the refrigerator coils as they warmed up and dried off. Swab samples were also taken directly from the coils.

The first experiments at Hotel "B" consisted of taking samples of air at 4 locations in the dehumidifying system. These results of 22 simultaneous collections are presented in table 5.

These results show an increase of 10.6 percent of the number of airborne microorganisms leaving the cooling coils as compared to the number in the return air, indicating an actual contamination of the air with airborne microorganisms.

TABLE 4

*These results indicate that Airborne Pathogenic Microorganisms are removed by the Kathabar*

	Recirculated air Station 1 (fig. 1)	Conditioned Air after fan Station 4 (fig. 1)	Fresh air to Kathabar Station 2 (fig. 1)	Air from Kathabar Station 3 (fig. 1)
Number of samples taken	21	21	21	21
Average No. of colonies	22	12	10	1 per 10 or 11 samples

TABLE 5

*Air samples taken from ducts at Hotel "B"*

	Fresh Air	Return Air	Air to Coils	Air from Coils
Number of samples taken	22	22	22	22
Average no. of colonies 10 ft. <sup>3</sup>	809	800	817	885

#### *Swab Tests*

In order to further check the increase in the number of microorganisms in the air leaving the refrigerator coils swabbings were made upon the surface of the fins of the refrigeration coil.

One square inch area of fin surface swabbed with a moistened sterile swab averaged more than 10,000 colonies when plated out on Tryptose Blood Agar and incubated at 22°C and 37°C for 48 to 96 hr.

#### *Growth and Release of Airborne Organism*

Since it now seemed apparent that the air leaving the refrigeration coils at hotel B contains more airborne microorganisms than the air to the coils and since it was found that there are large numbers of microorganisms on the coils the next experiment was designed to collect organisms from the air while the refrigeration was on and at approximately 10 min. intervals for as much as 1½ hr. after shutdown.

The data are presented in figure 2. It is apparent from the data that microorganisms are released in increasing numbers from refrigerator coils as they dry up, reaching a peak at about 53 min. after the refrigeration is shut off; thereafter declining again.

As a final check on the all glass impinger an experiment was designed to check the efficiency of the equipment by placing two impingers in series. The average results of 13 experiments which were conducted at hotel "A", are presented in table 6. The counts reported represent the number of microorganisms in 10 cu. ft. of air.

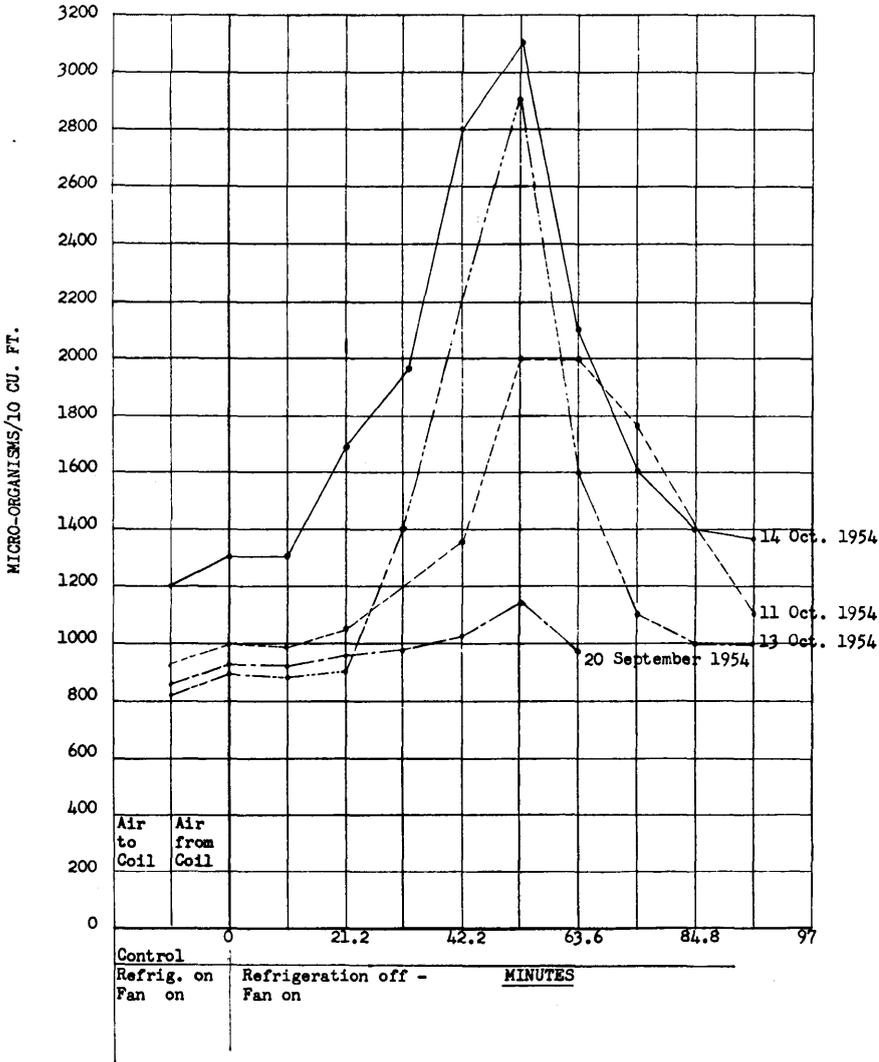


FIGURE 2. Graph showing numbers of airborne microorganisms collected from the air discharged through refrigerator coils at approximately 10 minute intervals after shut down.

TABLE 6

*A comparison of the number of Airborne Microorganisms removed by two All-Glass Impingers placed in series*

	First Sampler	Second Sampler	Percentage removed by Second Sampler
No. of tests	13	13	
Averages	232	2.4	1.03%

This table indicates that the all glass impinger is about 99 percent effective in the collection of airborne microorganisms.

## DISCUSSION

One of the most serious considerations in modern research on airborne microorganisms is the technique of collecting samples. Very misleading results may be obtained unless efficient techniques are used. Therefore, comparison of results reported herein to other published data should be made only with full consideration of the collection technique employed.

The efficiency of the all glass impinger has been checked by operating two units in series. Despite the physical difficulties of obtaining a high vacuum on both impingers it was possible to pull the same air through both impingers. Since only about 1 percent as many organisms were collected from the second impinger as compared to the first it is assumed that the first impinger has an efficiency of about 99 percent. Corrections can therefore be made on data in accordance with that efficiency, although this was not done in the present report.

The efficiency of the Luckiesh-Holladay-Taylor Electrostatic Sampler was also checked against the all glass impinger by taking simultaneous samples by means of the two methods. The average of 37 samples collected by both methods showed almost identical results with the two methods.

The efficiency of a funnel impinger technique is only about one tenth the efficiency of the electrostatic and impinger techniques. It is therefore unwise to rely upon data collected by this method unless consideration is given to its efficiency. Depending on "fall-out" it cannot be expected to have the efficiency of more positive collection methods.

It is difficult to compare the efficiencies of various pieces of equipment which are available for the removing of airborne organisms. The results of our experiments with the all glass impinger would indicate that impingement in an aqueous solution is a very efficient method. This is the method used in the Kathabar. However, Decker *et al.* (1954) have reported 99 percent efficiency for glass fiber media. For complete safety from pathogenic forms, an air incinerator was recommended.

In many situations there is need for complete removal of microorganisms from the air. As Jennings points out, in other situations a high percentage of removal is all that is required. The Kathabar system as presently designed will remove about 97 percent of the organisms (as corrected for the efficiency of the collecting system) and is therefore satisfactory for those situations requiring only a high percentage of removal. No doubt the efficiency of the Kathabar could be increased by redesign and by the addition of a second lithium chloride solution wash.

Several advantages of the Kathabar system of control of airborne microorganisms became apparent during the investigation. First of all, it is an automatic system requiring a minimum of maintenance. Second, it is not necessary to decontaminate the system if used to collect pathogenic forms because they are contained within the lithium chloride solution which is bactericidal. Third, the machine also controls humidity which is essential to human comfort and moisture sensitive industrial processes as well as to the control of microorganisms.

The removal of moisture in an air conditioning system by refrigeration is not adequate for the control of airborne microorganisms. The refrigeration system which was studied in the investigation reported herein did not remove microorganisms. In fact, the bacteria increased in numbers on the cooling coils, and were later released into the room air when the refrigeration system cycled.

## SUMMARY

The all glass impinger and the electrostatic air sampler were about equally effective in the collection of airborne microorganisms from air streams. The all glass impinger has an efficiency of about 99 percent. The electrostatic air sampler can then be presumed to have about the same efficiency.

Kathabar-treated air is relatively free of airborne organisms. This may be due to the impingement of organisms into the lithium chloride solution. The present data indicate that more than 97 percent of the organisms are removed by the Kathabar. The Kathabar was found to be as effective in the removal of airborne pathogenic microorganisms as in the removal of nonpathogenic forms.

Dehumidifying systems employing only refrigeration coils may increase the number of microorganisms in the air by providing on the refrigeration coils a suitable medium for the growth of microorganisms and their release into the air.

#### ACKNOWLEDGMENT

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