POTENTIAL INFECTIOUS HAZARDS OF COMMON BACTERIOLOGICAL TECHNIQUES

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A significant number of persons working with infectious microorganisms become infected. The resultant disease is often so mild that its specific nature is unrecognized, but in many cases the physical and economic consequences are serious.

A survey of reported bacterial infections acquired in the laboratory made by members of this installation, Stein and Segalove (1949), and included in a report by Sulkin and Pike (1950, 1951), and a similar survey of viral infections made by Sulkin and Pike (1949), revealed that many infections have resulted from accidents or poor techniques such as aspirating contaminated fluids into the mouth with a pipette, cuts from contaminated broken glassware, and pricking the skin with a hypodermic needle. In many of the published cases the mode of infection was unknown. Johansson and Ferris (1946) have shown photographically that aerosols are produced when the last drop is blown from a pipette. Stein, Anderson, and Gross (1949) and Anderson, Moss, and Gross (1950) have shown that standard laboratory techniques produce aerosols laden with bacteria which could serve as the infective dose in many unexplained laboratory infections.

By sampling the air during the performance of standard bacteriological techniques, a study was made to determine the extent of the escape of living organisms.

METHODS

The air samplers used were originally designed by DuBuy and Crisp (1944). The bacterial collecting efficiency of the sieve sampler is relatively high. Seventy-five to 80 per cent of all bacteria entering the sampler are impinged on the agar surface. The sieve sampler is a medium-impinging device and, therefore, will not give the number of bacteria in the air, because many bacteria in the air occur in clumps. The work of DuBuy, Hollaender, and Lackey (1945) seems to indicate that these clumps contain, on the average, about 10 organisms per clump. The sieve sampler consists of a cast aluminum alloy container (figure 1) in which is placed the bottom portion of an agar plate. Air was drawn through a sieve plate containing 300 holes at the rate of 1 cubic foot a minute, impinging the airborne organism upon the agar. The samplers were arranged to approximate the position of the hands, the position of the nose of the operator, and the space above and below the equipment used in the operations.

Serratia indica was used as the test organism in preference to *Serratia marcescens* because it pigmented more rapidly and produced fewer white variants. The organism never was found as an air contaminant in the controls. In most

cases the cultures were grown in a 250 ml Erlenmeyer flask containing 100 ml of nutrient broth and incubated for 24 hours at 25 C. This culture grown under these conditions consistently produced a count of 10^9 organisms per ml.

The medium used for the collection of air in the sampler plates was composed of processed corn steep liquor, 30 g; black strap molasses, 10 g; agar, 20 g; and distilled water, 1 liter. The medium then was adjusted to a pH of approximately 7.5. The test organism grew and pigmented exceptionally well on this medium. The techniques chosen for study were those most commonly used in bacteriological laboratories. An attempt was made to perform the techniques with normal care, but without exaggerating or minimizing the hazard. Several accidents that

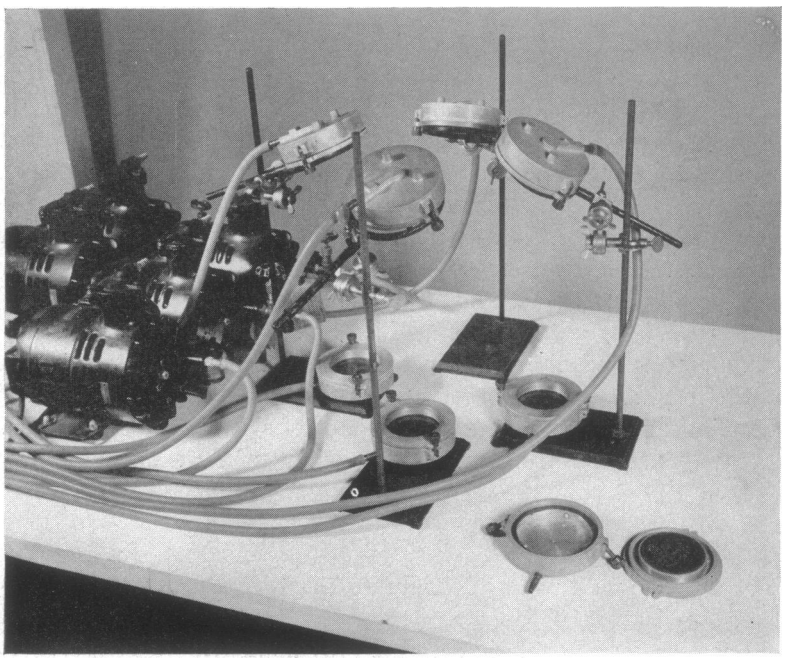

Figure 1. Sampling ensemble

might possibly occur also were investigated. Each operation was performed at least 15 times, and the average number of organisms recovered per unit operation was calculated. In some instances the operation was repeated 100 times.

The Waring Blendor. The Waring Blendor has become a standard item in many bacteriological laboratories. A study of the potential hazards created by the use of this apparatus was made. Two types of blending bowls were used; one was a one liter plastic capped bowl and the other was a 500 ml screw capped bowl. One 100 ml portion of a 24 hour broth culture of the test organism was blended for 2 minutes. Sieve samplers were operated during the blending procedure and for 3 minutes after the blendor was shut off. Considerable aerosol escaped from the capped bowls during this procedure. An aerosol of considerable magnitude (511

cells of *S. indica* per operation) was produced when the plastic capped bowl was used.

To illustrate the stability of the aerosol produced within the blendor bowl itself, the motor was turned off and the caps were removed immediately, after a 5 minute period, and after 1½ hours. In each case, 10 seconds after removal, the cap was replaced. An aerosol laden with 2,100 organisms per operation escaped from the bowl when either the plastic or screw cap was removed immediately.

TABLE 1

Serratia indica recovered from aerosols produced during operation of the Waring Blendor

Time of air sampling was 5 minutes in all experiments

	SERRATIA INDICA RECOVERED*	
	Plastic cap	Screw cap
During 2 min operation	511	18
Cap removed immediately after blendor was turned off	>2,100	>2,100
Cap removed 5 min after blendor was turned off	306	629
Cap removed 1½ hours after blendor was turned off	50	40

* Numbers refer to colonies appearing on sieve sampler plates.

TABLE 2

Serratia indica recovered from an aerosol produced by a drop of liquid culture falling on various surfaces

SURFACE	SERRATIA INDICA RECOVERED PER UNIT OPERATION	
	3 inches	12 inches
Stainless steel	8.0	48.0
Painted wood	1.4	43.0
"Kemrock"	0.2	26.0
Hand towel (dry)	0.8	28.0
Hand towel (phenol soaked)	0.1	3.7
Paper towel	0.7	0.8
Wrapping paper	0.1	2.5
Pan of phenol	0.0	0.1

The amount of aerosol liberated decreased as time progressed, but after 1½ hours there was still a considerable escape of organisms (table 1).

The falling drop. During pipetting a drop of culture medium sometimes falls to the bench top. The hazard involved when a drop falls on various surfaces was investigated. Twenty-five drops (approximately 1 ml) were allowed to fall from heights of 12 inches and 3 inches. The surfaces used were: stainless steel, painted wood, "kemrock" (a commercially prepared bench top), dry hand towel, paper towel, wrapping paper, hand towel wet with phenol, and a pan of phenol (5 per

cent). Sampling was continued only during the falling of the drops and for a few seconds thereafter.

The experiment showed that the type of surface markedly influenced the amount of aerosol produced. The most aerosol was produced when the drop fell 12 inches to the following surfaces: stainless steel (48 colonies), painted wood (43), "kemrock" (26), and a dry hand towel (28) (table 2).

Removal of stoppers from a shaken dilution bottle. Dilution bottles stoppered with various types of stoppers and caps were tested. Five ounce milk dilution bottles containing Escher stoppers, 8 ounce screw cap prescription bottles, and 250 ml Erlenmeyer flasks plugged with cotton were used.

One hundred ml of the culture was placed in each container and shaken. The bottles were shaken in an arc according to the recommendations for milk analysis, whereas the flask was shaken with a rotary motion to avoid wetting the plugs. Air samples were taken when the stoppers were removed immediately after shaking, and when the stoppers were removed after the containers had been allowed to sit for 30 seconds.

TABLE 3

Serratia indica recovered when stoppers were removed from dilution bottles that had been shaken

TYPE OF STOPPER	CONDITION OF STOPPER	SERRATIA INDICA RECOVERED PER UNIT OPERATION	
		Stopper removed immediately	Stopper removed after 30 sec delay
Escher stopper	wet	19.0	19.0
Screw cap	wet	8.0	11.0
Cotton plug	dry	1.4	0.6

Data in table 3 show that more bacteria escaped when an Escher stopper was removed from a bottle immediately after shaking than when a screw cap or cotton plug was used.

It appears that the aerosol created within the container was fairly stable. The counts obtained immediately after shaking and after a 30 second delay were of the same order of magnitude.

Removal of cotton plugs from culture tubes. Occasionally culture tubes are accidentally overturned. The hazard involved in the removal of wet plugs from these tubes was determined. The standard 16 mm culture test tube containing 5 ml of the standard broth culture of *S. indica* was tipped until the culture wet the base of the plug. The plug then was removed and the aerosol determined.

Data in table 4 show that a considerable number of organisms escaped into the air. The removal of a dry cotton plug from an unshaken tube of similar size caused no demonstrable escape of organisms as shown in the controls. However, when a 15 ml centrifuge tube containing 10 ml culture was used, an aerosol (2.5 cells of *S. indica* per unit operation) was created when the dry plug was removed (table 4, uncentrifuged tubes). The process of centrifugation apparently created an aerosol within the tubes because a greater number of organisms (6.4 cells of

S. indica per unit operation) was recovered when the plugs were removed following this operation.

Removal of an inoculum from a vaccine bottle with a hypodermic syringe. The transfer of pathogenic cultures by use of a hypodermic syringe sometimes has been considered one of the safest methods used in the laboratory. Some investigators believe that the contents of the rubber capped vaccine bottle should be under reduced atmospheric pressure when the sample is removed while others feel that a considerable volume of air should be injected prior to removal of the sample.

TABLE 4

Serratia indica recovered when cotton plugs were removed from test tubes

OPERATION	SERRATIA INDICA PER UNIT OPERATION
Test tube (16 mm) with 5 ml of culture	
Control	0.0
Wetted plug removed normally	10.2
Wetted plug removed carefully	6.8
Centrifuge tube (15 ml) with 10 ml of culture	
Uncentrifuged tube	2.5
Centrifuged tube	6.4

TABLE 5

Serratia indica recovered during removal of an inoculum from a vaccine bottle

TREATMENT OF VACCINE BOTTLE PRIOR TO REMOVAL OF SAMPLE	SERRATIA INDICA PER UNIT OPERATION	
	With pledget	Without pledget
No treatment	0.2	0.6
Atmospheric pressure	0.1	0.2
Negative pressure	0.5	0.5
Positive pressure	0.6	1.7

Various pressure treatments were applied to the vaccine bottle in investigation of this transfer technique. Atmospheric pressure was produced by first inserting the needle only and allowing air pressure to equilibrate. Negative pressure was produced by withdrawing a quantity of air with a syringe and removing the needle. Positive pressure was obtained by forcing air into the bottle with a syringe.

An attempt to minimize the aerosol produced was done by placing a cotton pledget soaked in alcohol around the needle next to the rubber cap on the vaccine bottle while the needle was removed.

Table 5 indicates that an aerosol was created when an inoculum was removed from a vaccine bottle with a hypodermic syringe and that the use of a cotton pledget, soaked in alcohol, helped to reduce it. High speed photography revealed

that the needle on the hypodermic syringe vibrated considerably when withdrawn from the rubber stopper and droplets containing microorganisms were thrown into the air.

Flaming an inoculating loop. The process of flaming an inoculating loop has long been suspected of being a hazardous bacteriological procedure. When an inoculum of *S. indica* was removed by inserting a hot loop in a broth culture contained in a 250 ml Erlenmeyer flask and flamed in a Fisher burner, 8.7 organisms were recovered per flaming as shown in table 6.

If the same process was repeated except that the inoculum was removed from a test tube instead of a flask, a negligible number of organisms was recovered (0.1 cells per operation). This indicated that the organisms were escaping from the container rather than being spread by splattering during the actual flaming. When care was taken to cool the loop before inserting it into the culture, it was possible to reduce the number of *S. indica* recovered to 0.8 per flaming operation for the 250 ml flask.

TABLE 6

Serratia indica recovered during the flaming of an inoculum loop

METHOD OF INSERTING INOCULATION LOOP	SERRATIA INDICA PER UNIT OPERATION	
	Broth culture in test tube	Broth culture in 250 ml flask
Hot loop	0.1	8.7
Cold loop	0.1	0.8

A study was made of the flaming techniques used by several individuals. The inoculum was taken from a 250 ml flask. There was considerable variation in the number of organisms recovered when the same technique was performed by various persons, varying from 6 organisms per flaming to 24. Those whose techniques produced the greater aerosols inserted the hot loop into the culture, shook the loop in the culture, or moved the flask as the loop was inserted.

Inoculating techniques: transfer of inoculum with a pipette. Frequently before transferring an inoculum from a broth culture with a pipette, a worker will mix the culture by alternate suction and blowing on the pipette. When blowing was done to produce bubbling, 0.8 cells of *S. indica* per unit operation was recovered per culture mixed. If the blowing was done without producing bubbling, the number recovered was reduced to 0.2 as shown in table 7.

The number of airborne *S. indica* recovered while pipetting a 1 ml inoculum from a culture tube to a 250 ml flask containing 100 ml of broth was 1.2. When 10 ml of inoculum were transferred to a 2 liter flask containing 1 liter of broth, 2.4 airborne *S. indica* were recovered. Care was taken to avoid splashing, and the culture was carefully run down the side of the flask.

Vibrating inoculating loop. Occasionally while withdrawing an inoculum from a culture tube with a wire loop, the loop will accidentally touch the side of the tube and vibrate. When this was done intentionally, 0.6 airborne *S. indica* was recovered in an average operation.

Bursting of the film on an inoculating loop. The film held in an inoculating loop has been observed to burst. When this was done intentionally by touching it with an inoculating needle, 0.2 *S. indica* was recovered per film burst (table 7).

Streaking an agar plate with a loop of culture from an agar colony and from a broth culture. The techniques of streaking an agar plate with a loop inoculum taken from an agar colony and from a broth culture were investigated. The data (table 7) obtained indicated little escape of organisms by these procedures. There was less than 0.1 *S. indica* recovered per unit operation when the inoculum was taken from the agar colony and 0.2 when the broth inoculum was employed. The fact that some organisms were recovered indicated that under certain conditions, such as rough agar surface which may cause the needle to vibrate, an appreciable aerosol may be created.

TABLE 7

Serratia indica recovered during various inoculating techniques

INOCULATING TECHNIQUE	SERRATIA INDICA PER UNIT OPERATION
Mixing culture with bubbling	0.8
Mixing culture without bubbling	0.2
Transfer of 1 ml of inoculum	1.2
Transfer of 10 ml of inoculum	2.4
Vibrating inoculating loop	0.6
Bursting film of inoculating loop	0.2
Streaking agar plate from agar colony	0.1
Streaking agar plate from broth	0.2

DISCUSSION

The most hazardous laboratory procedures seem to be those in which splashing or bubbling occurs. Probably the most commonly encountered is the shaking of liquid cultures. When the bubbles burst they release into the air bacteria-laden aerosols of sufficiently small particle size so that they remain suspended for some time. When any such culture is opened, the organisms will escape into the surrounding air.

The hazard involved in opening shaken dilution bottles is partially alleviated by use of Erlenmeyer flasks and by rotating them instead of shaking them up and down. Several counts, made from dilution flasks agitated by rotation, revealed no significant difference from counts obtained after shaking in prescription bottles or milk dilution bottles. A part of the aerosol released from prescription bottles and the milk dilution bottles probably is produced when the film around the caps or stoppers is broken as the cap or stopper is removed. Plastic caps, after having been autoclaved several times, become warped and cracked and are likely to leak, thus creating an additional danger. From these

observations it is recommended that, when highly infectious agents are in use, all dilutions be made in a ventilated bacteriological cabinet.

The Waring Blendor creates an aerosol highly laden with bacteria. Such substances as embryonic material containing egg albumin form foams which may persist for several hours. With the present design of the Waring Blendor it seems impossible to prevent leaks. It, therefore, seems advisable to use the blendor in a ventilated bacteriological cabinet in which the outside of the blendor can be decontaminated before removal from the cabinet.

Even careful laboratory workers may occasionally allow a drop of culture medium to fall during pipetting. If the drop falls on a hard nonabsorbent surface, aerosols are formed. Many workers use a towel for a working surface believing that its absorbent quality will prevent splattering of falling drops. It was observed under a dissecting microscope that when a drop fell on a towel there was considerable rapid movement of the cotton fibers, which persisted for some seconds after the drop had fallen. This is especially true when the drop fell on a dry hand towel. It is presumed that this is the reason for the unexpectedly large amount of aerosol which was recovered by air sampling.

The practice of removing wet plugs from culture tubes should be avoided. The use of small or compartmented baskets will aid in preventing tubes from tipping and wetting the plugs. In the centrifuge, aside from breaking tubes, there is an important danger point at the opening of the centrifuged tubes. This seems to be due partly to the fact that there is a small amount of foaming and the plugs become slightly moistened. This can be minimized by filling the tubes only half full. It is best to open all liquid cultures in a ventilated cabinet.

Much of the aerosol released during the removal of an inoculum from a vaccine bottle can be prevented by use of an alcohol-soaked cotton pledget around the hypodermic needle; however, this does not solve the problem completely. High speed motion pictures have revealed that the vibration of the needle as the tip emerges from the diaphragm produces an aerosol (Stein, Anderson, and Gross, 1949).

The number of organisms liberated into the air during flaming of a loop can be reduced by making sure that the loop is cool before inserting in into the culture, by not shaking the flask, and by not stirring the culture with the loop.

In the experiments, considerable variation was observed in the results obtained from one experiment to another; therefore, the experiments were repeated at least 15 times and the average per unit operation was recorded. The results are more qualitative than quantitative in that only the order of magnitude of bacteria-laden aerosol was established. Variations in temperature, humidity, air currents, and the amount of dust probably contributed to the variation observed. An attempt was made to keep the procedures standard and not to exaggerate them in any way. When no organisms were recovered, it cannot be assumed that organisms were not released; the samplers are considered to be about 75 to 80 per cent efficient in impinging the organisms entering the sampler. In procedures where large numbers were recovered the operation must be considered hazardous.

SUMMARY

Aerosols laden with bacteria in sufficient numbers to infect laboratory workers are released during the performance of many common laboratory techniques.

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